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(54) Title: PESTICIDAL TOXINS

(57) Abstract

The subject invention concerns new classes of pesticidal toxins and polynucleotide sequences which encode these toxins. Also described are novel pesticidal isolates of *Bacillus thuringiensis*.

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DESCRIPTION

PESTICIDAL TOXINS

Cross-Reference to a Related Application

This application is a continuation-in-part of Application Serial No. 08/633,993, filed April 19, 1996.

Background of the Invention

The soil microbe *Bacillus thuringiensis* (*B.t.*) is a Gram-positive, spore-forming bacterium characterized by parasporal crystalline protein inclusions. These inclusions often appear microscopically as distinctively shaped crystals. The proteins can be highly toxic to pests and specific in their toxic activity. Certain *B.t.* toxin genes have been isolated and sequenced, and recombinant DNA-based *B.t.* products have been produced and approved for use. In addition, with the use of genetic engineering techniques, new approaches for delivering these *B.t.* endotoxins to agricultural environments are under development, including the use of plants genetically engineered with endotoxin genes for insect resistance and the use of stabilized intact microbial cells as *B.t.* endotoxin delivery vehicles (Gaertner, F.H., L. Kim [1988] *TIBTECH* 6:S4-S7). Thus, isolated *B.t.* endotoxin genes are becoming commercially valuable.

Until the last ten years, commercial use of *B.t.* pesticides has been largely restricted to a narrow range of lepidopteran (caterpillar) pests. Preparations of the spores and crystals of *B. thuringiensis* subsp. *kurstaki* have been used for many years as commercial insecticides for lepidopteran pests. For example, *B. thuringiensis* var. *kurstaki* HD-1 produces a crystalline δ-endotoxin which is toxic to the larvae of a number of lepidopteran insects.

In recent years, however, investigators have discovered *B.t.* pesticides with specificities for a much broader range of pests. For example, other species of *B.t.*, namely *israelensis* and *tenebrionis* (a.k.a. *B.t.* M-7, a.k.a. *B.t. san diego*), have been used commercially to control insects of the orders Diptera and Coleoptera, respectively (Gaertner, F.H. [1989] "Cellular Delivery Systems for Insecticidal Proteins: Living and Non-Living Microorganisms," in *Controlled Delivery of Crop Protection Agents*, R.M. Wilkins, ed., Taylor and Francis, New York and London, 1990, pp. 245-255). See also Couch, T.L. (1980) "Mosquito Pathogenicity of *Bacillus thuringiensis* var. *israelensis*," *Developments in Industrial Microbiology* 22:61-76; Beegle, C.C., (1978) "Use of Entomogenous Bacteria in Agroecosystems," *Developments in Industrial Microbiology* 20:97-104. Krieg, A., A.M. Huger, G.A. Langenbruch, W. Schnetter

(1983) *Z. ang. Ent.* 96:500-508, describe *Bacillus thuringiensis* var. *tenebrionis*, which is reportedly active against two beetles in the order Coleoptera. These are the Colorado potato beetle, *Leptinotarsa decemlineata*, and *Agelastica alni*.

Recently, new subspecies of *B.t.* have been identified, and genes responsible for active δ-endotoxin proteins have been isolated (Höfte, H., H.R. Whiteley [1989] *Microbiological Reviews* 52(2):242-255). Höfte and Whiteley classified *B.t.* crystal protein genes into 4 major classes. The classes were CryI (Lepidoptera-specific), CryII (Lepidoptera- and Diptera-specific), CryIII (Coleoptera-specific), and CryIV (Diptera-specific). The discovery of strains specifically toxic to other pests has been reported. (Feitelson, J.S., J. Payne, L. Kim [1992] *Bio/Technology* 10:271-275).

The cloning and expression of a *B.t.* crystal protein gene in *Escherichia coli* has been described in the published literature (Schnepf, H.E., H.R. Whiteley [1981] *Proc. Natl. Acad. Sci. USA* 78:2893-2897). U.S. Patent 4,448,885 and U.S. Patent 4,467,036 both disclose the expression of *B.t.* crystal protein in *E. coli*. U.S. Patents 4,797,276 and 4,853,331 disclose *B. thuringiensis* strain *tenebrionis* (a.k.a. M-7, a.k.a. *B.t. san diego*) which can be used to control coleopteran pests in various environments. U.S. Patent No. 4,918,006 discloses *B.t.* toxins having activity against Dipterans. U.S. Patent No. 4,849,217 discloses *B.t.* isolates which have activity against the alfalfa weevil. U.S. Patent No. 5,208,077 discloses coleopteran-active *Bacillus thuringiensis* isolates. U.S. Patent No. 5,151,363 and U.S. Patent No. 4,948,734 disclose certain isolates of *B.t.* which have activity against nematodes. As a result of extensive research and investment of resources, other patents have issued for new *B.t.* isolates and new uses of *B.t.* isolates. However, the discovery of new *B.t.* isolates and new uses of known *B.t.* isolates remains an empirical, unpredictable art.

Coleopterans are an important group of agricultural pests which cause a very large amount of damage each year. Examples of coleopteran pests include alfalfa weevils and corn rootworm.

The alfalfa weevil, *Hypera postica*, and the closely related Egyptian alfalfa weevil, *Hypera brunneipennis*, are the most important insect pests of alfalfa grown in the United States, with 2.9 million acres infested in 1984. An annual sum of 20 million dollars is spent to control these pests. The Egyptian alfalfa weevil is the predominant species in the southwestern U.S., where it undergoes aestivation (*i.e.*, hibernation) during the hot summer months. In all other respects, it is identical to the alfalfa weevil, which predominates throughout the rest of the U.S.

The larval stage is the most damaging in the weevil life cycle. By feeding at the alfalfa plant's growing tips, the larvae cause skeletonization of leaves, stunting, reduced plant growth,

and, ultimately, reductions in yield. Severe infestations can ruin an entire cutting of hay. The adults, also foliar feeders, cause additional, but less significant, damage.

Approximately 9.3 million acres of U.S. corn are infested with corn rootworm species complex each year. The corn rootworm species complex includes the northern corn rootworm, *Diabrotica barberi*, the southern corn rootworm, *D. undecimpunctata howardi*, and the western corn rootworm, *D. virgifera virgifera*. The soil-dwelling larvae of these *Diabrotica* species feed on the root of the corn plant, causing lodging. Lodging eventually reduces corn yield and often results in death of the plant. By feeding on cornsilks, the adult beetles reduce pollination and, therefore, detrimentally effect the yield of corn per plant. In addition, adults and larvae of the genus *Diabrotica* attack cucurbit crops (cucumbers, melons, squash, etc.) and many vegetable and field crops in commercial production as well as those being grown in home gardens.

Control of corn rootworm has been partially addressed by cultivation methods, such as crop rotation and the application of high nitrogen levels to stimulate the growth of an adventitious root system. However, chemical insecticides are relied upon most heavily to guarantee the desired level of control. Insecticides are either banded onto or incorporated into the soil. The major problem associated with the use of chemical insecticides is the development of resistance among the treated insect populations.

Brief Summary of the Invention

The subject invention concerns novel materials and methods for controlling non-mammalian pests. In a preferred embodiment, the subject invention provides materials and methods for the control of coleopteran pests. In specific embodiments, the materials and methods described herein are used to control alfalfa weevil and/or corn rootworm.

The subject invention advantageously provides two new classes of polynucleotide sequences which encode corresponding novel classes of pesticidal proteins. One novel class of polynucleotide sequences as described herein encodes toxins which have a full-length molecular weight of approximately 40-50 kDa. In a specific embodiment, these toxins have a molecular weight of about 43-47 kDa. A second class of polynucleotides, which encodes pesticidal proteins of about 10-15 kDa, is also provided according to the subject invention. In a specific embodiment, these toxins have a molecular weight of about 13-14 kDa. The subject invention concerns polynucleotides which encode the 40-50 kDa and 10-15 kDa toxins, polynucleotides which encode pesticidal fragments of the full length toxins, and polynucleotide sequences which encode longer forms of these toxins which include, for example, a protoxin region. In a

preferred embodiment, these toxins, including the fragments, are active against coleopteran pests.

Specific *B.t.* toxins useful according to the invention include toxins which can be obtained from the *B.t.* isolates designated as PS80JJ1, PS149B1, and PS167H2. Of these, PS149B1 and PS167H2 are novel isolates. The subject invention also includes the use of variants of the exemplified *B.t.* isolates and toxins which have substantially the same coleopteran-active properties as the specifically exemplified *B.t.* isolates and toxins. Such variant isolates would include, for example, mutants. Procedures for making mutants are well known in the microbiological art. Ultraviolet light and chemical mutagens such as nitrosoguanidine are used extensively toward this end.

In one embodiment of the subject invention, the polynucleotide sequences of the subject invention are used to encode toxins of approximately 43-47 kDa. These toxins are then used to control coleopteran pests. In a particularly preferred embodiment, the coleopteran pests are corn rootworms. The genes which encode the 43-47 kDa toxins can be obtained from, for example, PS80JJ1, PS149B1, or PS167H2. In a second embodiment, toxins of approximately 13-14 kDa are used to control coleopteran pests. The approximately 13-14 kDa toxin, as well as the genes which encode these toxins, can also be obtained from PS80JJ1, PS149B1, or PS167H2. In a particularly preferred embodiment, the activity of the 43-47 kDa toxins can be augmented and/or facilitated by further contacting the target pests with an approximately 13-14 kDa toxin.

In a preferred embodiment, the subject invention concerns plants cells transformed with at least one polynucleotide sequence of the subject invention such that the transformed plant cells express pesticidal toxins in tissues consumed by the target pests.

Alternatively, the *B.t.* isolates of the subject invention, or recombinant microbes expressing the toxins described herein, can be used to control pests. In this regard, the invention includes the treatment of substantially intact *B.t.* cells, and/or recombinant cells containing the expressed toxins of the invention, treated to prolong the pesticidal activity when the substantially intact cells are applied to the environment of a target pest. The treated cell acts as a protective coating for the pesticidal toxin. The toxin becomes active upon ingestion by a target insect.

Brief Description of the Drawings

Figure 1 shows three specific 43-47 kDa pesticidal toxins of the subject invention as well as a consensus sequence for these pesticidal toxins.

Figure 2 shows the relationship of the 14 and 45 kDa sequences of PS80JJ1 (SEQ ID NOS. 31 and 10).

Brief Description of the Sequences

SEQ ID NO. 1 is a 5-amino acid N-terminal sequence of the approximately 45 kDa toxin of 80JJ1.

SEQ ID NO. 2 is a 25-amino acid N-terminal sequence of the approximately 45 kDa toxin of 80JJ1.

SEQ ID NO. 3 is a 24-amino acid N-terminal sequence of the approximately 14 kDa toxin of 80JJ1.

SEQ ID NO. 4 is the N-terminal sequence of the approximately 47 kDa toxin from 149B1.

SEQ ID NO. 5 is a 50-amino acid N-terminal amino acid sequence for the purified approximately 14 kDa protein from PS149B1.

SEQ ID NO. 6 is the N-terminal sequence of the approximately 47 kDa toxin from 167H2.

SEQ ID NO. 7 is a 25-amino acid N-terminal sequence for the purified approximately 14 kDa protein from PS167H2.

SEQ ID NO. 8 is an oligonucleotide probe for the gene encoding the PS80JJ1 44.3 kDa toxin and is a forward primer for PS149B1 and PS167H2 used according to the subject invention.

SEQ ID NO. 9 is a reverse primer for PS149B1 and PS167H2 used according to the subject invention.

SEQ ID NO. 10 is the nucleotide sequence of the gene encoding the approximately 45 kDa PS80JJ1 toxin.

SEQ ID NO. 11 is the amino acid sequence for the approximately 45 kDa PS80JJ1 toxin.

SEQ ID NO. 12 is the partial nucleotide sequence of the gene encoding the approximately 44 kDa PS149B1 toxin.

SEQ ID NO. 13 is the partial amino acid sequence for the approximately 44 kDa PS149B1 toxin.

SEQ ID NO. 14 is the partial nucleotide sequence of the gene encoding the approximately 44 kDa PS167H2 toxin.

SEQ ID NO. 15 is the partial amino acid sequence for the approximately 44 kDa PS167H2 toxin.

SEQ ID NO. 16 is a peptide sequence used in primer design according to the subject invention.

SEQ ID NO. 17 is a peptide sequence used in primer design according to the subject invention.

SEQ ID NO. 18 is a peptide sequence used in primer design according to the subject invention.

SEQ ID NO. 19 is a peptide sequence used in primer design according to the subject invention.

SEQ ID NO. 20 is a nucleotide sequence corresponding to the peptide of SEQ ID NO. 16.

SEQ ID NO. 21 is a nucleotide sequence corresponding to the peptide of SEQ ID NO. 17.

SEQ ID NO. 22 is a nucleotide sequence corresponding to the peptide of SEQ ID NO. 18.

SEQ ID NO. 23 is a nucleotide sequence corresponding to the peptide of SEQ ID NO. 19.

SEQ ID NO. 24 is a reverse primer based on the reverse complement of SEQ ID NO. 22.

SEQ ID NO. 25 is a reverse primer based on the reverse complement of SEQ ID NO. 23.

SEQ ID NO. 26 is a forward primer based on the PS80JJ1 44.3 kDa toxin.

SEQ ID NO. 27 is a reverse primer based on the PS80JJ1 44.3 kDa toxin.

SEQ ID NO. 28 is a generic sequence representing a new class of toxins according to the subject invention.

SEQ ID NO. 29 is an oligonucleotide probe used according to the subject invention.

SEQ ID NO. 30 is the nucleotide sequence of the entire genetic locus containing open reading frames of both the 14 and 45 kDa PS80JJ1 toxins and the flanking nucleotide sequences.

SEQ ID NO. 31 is the nucleotide sequence of the PS80JJ1 14 kDa toxin open reading frame.

SEQ ID NO. 32 is the deduced amino acid sequence of the 14 kDa toxin of PS80JJ1.

SEQ ID NO. 33 is a reverse oligonucleotide primer used according to the subject invention.

SEQ ID NO. 34 is the nucleotide sequence of the entire genetic locus containing open reading frames of both the 14 and 44 kDa PS167H2 toxins and the flanking nucleotide sequences.

SEQ ID NO. 35 is the nucleotide sequence of the gene encoding the approximately 14 kDa PS167H2 toxin.

SEQ ID NO. 36 is the amino acid sequence for the approximately 14 kDa PS167H2 toxin.

SEQ ID NO. 37 is the nucleotide sequence of the gene encoding the approximately 44 kDa PS167H2 toxin.

SEQ ID NO. 38 is the amino acid sequence for the approximately 44 kDa PS167H2 toxin.

SEQ ID NO. 39 is the nucleotide sequence of the entire genetic locus containing open reading frames of both the 14 and 44 kDa PS149B1 toxins and the flanking nucleotide sequences.

SEQ ID NO. 40 is the nucleotide sequence of the gene encoding the approximately 14 kDa PS149B1 toxin.

SEQ ID NO. 41 is the amino acid sequence for the approximately 14 kDa PS149B1 toxin.

SEQ ID NO. 42 is the nucleotide sequence of the gene encoding the approximately 44 kDa PS149B1 toxin.

SEQ ID NO. 43 is the amino acid sequence for the approximately 44 kDa PS149B1 toxin.

SEQ ID NO. 44 is a maize-optimized gene sequence encoding the approximately 14 kDa toxin of 80JJ1.

SEQ ID NO. 45 is a maize-optimized gene sequence encoding the approximately 44 kDa toxin of 80JJ1.

Detailed Disclosure of the Invention

The subject invention concerns two new classes of polynucleotide sequences which encode novel pesticidal toxins. In one embodiment, the toxins have a full-length molecular weight of approximately 40-50 kDa. In specific embodiments exemplified herein, these toxins have a molecular weight of about 43-47 kDa. In a second embodiment, the pesticidal toxins have a molecular weight of approximately 10-15 kDa. In specific embodiments exemplified herein, these toxins have a molecular weight of about 13-14 kDa. Certain specific toxins are exemplified herein. For toxins having a known amino acid sequence, the molecular weight is also known. Those skilled in the art will recognize that the apparent molecular weight of a protein as determined by gel electrophoresis will sometimes differ from the true molecular

weight. Therefore, reference herein to, for example, a 45 kDa protein or a 14 kDa protein is understood to refer to proteins of approximately that size even if the true molecular weight is somewhat different.

The subject invention concerns not only the polynucleotide sequences which encode these classes of toxins, but also the use of these polynucleotide sequences to produce recombinant hosts which express the toxins. In a further aspect, the subject invention concerns the combined use of an approximately 40-50 kDa toxin of the subject invention together with an approximately 10-15 kDa toxin of the subject invention to achieve highly effective control of pests, including coleopterans such as corn rootworm.

A further aspect of the subject invention concerns two novel isolates and the toxins and genes obtainable from these isolates. The novel *B.t.* isolates of the subject invention have been designated PS149B1 and PS167H2.

The new classes of toxins and polynucleotide sequences provided here are defined according to several parameters. One critical characteristic of the toxins described herein is pesticidal activity. In a specific embodiment, these toxins have activity against coleopteran pests. The toxins and genes of the subject invention can be further defined by their amino acid and nucleotide sequences. The sequences of the molecules within each novel class can be defined herein in terms of homology to certain exemplified sequences as well as in terms of the ability to hybridize with, or be amplified by, certain exemplified probes and primers. The classes of toxins provided herein can also be identified based on their immunoreactivity with certain antibodies and based upon their adherence to a generic formula.

The sequence of three approximately 45 kDa toxins of the subject invention are provided as SEQ ID NOS. 11, 43, and 38. In a preferred embodiment of the subject invention, the toxins in this new class have a sequence which conforms to the generic sequence presented as SEQ ID NO. 28. In a specific embodiment, the toxins of this class will conform to the consensus sequence shown in Figure 1.

In a preferred embodiment, the toxins of the subject invention have at least one of the following characteristics:

(a) said toxin is encoded by a nucleotide sequence which hybridizes under stringent conditions with a nucleotide sequence selected from the group consisting of: DNA which encodes SEQ ID NO. 2, DNA which encodes SEQ ID NO. 4, DNA which encodes SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, DNA which encodes SEQ ID NO. 11, SEQ ID NO. 12, DNA which encodes SEQ ID NO. 13, SEQ ID NO. 14, DNA which encodes SEQ ID NO. 15, DNA which encodes

SEQ ID NO. 16, DNA which encodes SEQ ID NO. 17, DNA which encodes SEQ ID NO. 18, DNA which encodes SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, DNA which encodes a pesticidal portion of SEQ ID NO. 28, SEQ ID NO. 37, DNA which encodes SEQ ID NO. 38, SEQ ID NO. 42, and DNA which encodes SEQ ID NO. 43;

(b) said toxin immunoreacts with an antibody to an approximately 40-50 kDa pesticidal toxin, or a fragment thereof, from a *Bacillus thuringiensis* isolate selected from the group consisting of PS80JJ1 having the identifying characteristics of NRRL B-18679, PS149B1 having the identifying characteristics of NRRL B-21553, and PS167H2 having the identifying characteristics of NRRL B-21554;

(c) said toxin is encoded by a nucleotide sequence wherein a portion of said nucleotide sequence can be amplified by PCR using a primer pair selected from the group consisting of SEQ ID NOS. 20 and 24 to produce a fragment of about 495 bp, SEQ ID NOS. 20 and 25 to produce a fragment of about 594 bp, SEQ ID NOS. 21 and 24 to produce a fragment of about 471 bp, and SEQ ID NOS. 21 and 25 to produce a fragment of about 580 bp;

(d) said toxin comprises a pesticidal portion of the amino acid sequence shown in SEQ ID NO. 28;

(e) said toxin comprises an amino acid sequence which has at least about 60% homology with a pesticidal portion of an amino acid sequence selected from the group consisting of SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 38, and SEQ ID NO. 43;

(f) said toxin is encoded by a nucleotide sequence which hybridizes under stringent conditions with a nucleotide sequence selected from the group consisting of DNA which encodes SEQ ID NO. 3, DNA which encodes SEQ ID NO. 5, DNA which encodes SEQ ID NO. 7, DNA which encodes SEQ ID NO. 32, DNA which encodes SEQ ID NO. 36, and DNA which encodes SEQ ID NO. 41;

(g) said toxin immunoreacts with an antibody to an approximately 10-15 kDa pesticidal toxin, or a fragment thereof, from a *Bacillus thuringiensis* isolate selected from the group consisting of PS80JJ1 having the identifying characteristics of NRRL B-18679, PS149B1 having the identifying

characteristics of NRRL B-21553, and PS167H2 having the identifying characteristics of NRRL B-21554;

(h) said toxin is encoded by a nucleotide sequence wherein a portion of said nucleotide sequence can be amplified by PCR using the primer pair of SEQ ID NO. 29 and SEQ ID NO. 33; and

(i) said toxin comprises an amino acid sequence which has at least about 60% homology with an amino acid sequence selected from the group consisting of SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of SEQ ID NO. 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of SEQ ID NO. 41.

As used herein "stringent" conditions for hybridization refers to conditions which achieve the same, or about the same, degree of specificity of hybridization as the conditions employed by the current applicants. Specifically, hybridization of immobilized DNA on Southern blots with 32P-labeled gene-specific probes was performed by standard methods (Maniatis, T., E.F. Fritsch, J. Sambrook [1982] *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.). In general, hybridization and subsequent washes were carried out under stringent conditions that allowed for detection of target sequences with homology to the PS80JJ1 toxin genes. For double-stranded DNA gene probes, hybridization was carried out overnight at 20-25° C below the melting temperature (Tm) of the DNA hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature is described by the following formula (Beltz, G.A., K.A. Jacobs, T.H. Eickbush, P.T. Cherbas, and F.C. Kafatos [1983] *Methods of Enzymology*, R. Wu, L. Grossman and K. Moldave [eds.] Academic Press, New York 100:266-285).

Tm=81.5° C+16.6 Log[Na+]+0.41(%G+C)-0.61(%formamide)-600/length of duplex in base pairs.

Washes are typically carried out as follows:

(1) Twice at room temperature for 15 minutes in 1X SSPE, 0.1% SDS (low stringency wash).

(2) Once at Tm-20°C for 15 minutes in 0.2X SSPE, 0.1% SDS (moderate stringency wash).

For oligonucleotide probes, hybridization was carried out overnight at 10-20°C below the melting temperature (Tm) of the hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. Tm for oligonucleotide probes was determined by the following formula:

Tm (° C)=2(number T/A base pairs) +4(number G/C base pairs)

(Suggs, S.V., T. Miyake, E.H. Kawashime, M.J. Johnson, K. Itakura, and R.B. Wallace [1981] *ICN-UCLA Symp. Dev. Biol. Using Purified Genes*, D.D. Brown [ed.], Academic Press, New York, 23:683-693).

Washes were typically carried out as follows:

(1) Twice at room temperature for 15 minutes 1X SSPE, 0.1% SDS (low stringency wash).

(2) Once at the hybridization temperature for 15 minutes in 1X SSPE, 0.1% SDS (moderate stringency wash).

With the teachings provided herein, one skilled in the art could readily produce and use the various toxins and polynucleotide sequences of the novel classes described herein.

Microorganisms useful according to the subject invention have been deposited in the permanent collection of the Agricultural Research Service Patent Culture Collection (NRRL), Northern Regional Research Center, 1815 North University Street, Peoria, Illinois 61604, USA. The culture repository numbers of the deposited strains are as follows:

Culture	Repository No.	Deposit Date
B.t. strain PS80JJ1	NRRL B-18679	July 17, 1990
B.t. strain PS149B1	NRRL B-21553	March 28, 1996
B.t. strain PS167H2	NRRL B-21554	March 28, 1996
E. coli NM522 (pMYC2365)	NRRL B-21170	January 5, 1994
E. coli NM522 (pMYC2382)	NRRL B-21329	September 28, 1994
E. coli NM522 (pMYC2379)	NRRL B-21155	November 3, 1993
E. coli NM522(pMYC2421)	NRRL B-21555	March 28, 1996
E. coli NM522(pMYC2427)	NRRL B-21672	March 26, 1997
E. coli NM522(pMYC2429)	NRRL B-21673	March 26, 1997
E. coli NM522(pMYC2426)	NRRL B-21671	March 26, 1997

The PS80JJ1 isolate is available to the public by virtue of the issuance of U.S. Patent No. 5,151,363.

B.t. isolates PS149B1 and PS167H2 have been deposited under conditions that assure that access to the cultures will be available during the pendency of this patent application to one determined by the Commissioner of Patents and Trademarks to be entitled thereto under 37 CFR 1.14 and 35 U.S.C. 122. The deposits are available as required by foreign patent laws in countries wherein counterparts of the subject application, or its progeny, are filed. However, it

should be understood that the availability of a deposit does not constitute a license to practice the subject invention in derogation of patent rights granted by governmental action.

Further, the subject culture deposits will be stored and made available to the public in accord with the provisions of the Budapest Treaty for the Deposit of Microorganisms, i.e., they will be stored with all the care necessary to keep them viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of a deposit, and in any case, for a period of at least 30 (thirty) years after the date of deposit or for the enforceable life of any patent which may issue disclosing the cultures. The depositor acknowledges the duty to replace the deposit(s) should the depository be unable to furnish a sample when requested, due to the condition of the deposit(s). All restrictions on the availability to the public of the subject culture deposits will be irrevocably removed upon the granting of a patent disclosing them.

Following is a table which provides characteristics of certain *B.t.* isolates useful according to the subject invention.

Table 1. Description of *B.t.* strains toxic to coleopterans

Culture	Crystal Description	Approx. MW (kDa)	Serotype	NRRL Deposit	Deposit Date
PS80JJ1	multiple attached	130, 90, 47, 37, 14	4a4b, sotto	B-18679	7-17-90
PS149B1		130, 47, 14		B-21553	3-28-96
PS167H2		70, 47, 14		B-23554	3-28-96

Genes and toxins. The genes and toxins useful according to the subject invention include not only the full length sequences disclosed but also fragments of these sequences, variants, mutants, and fusion proteins which retain the characteristic pesticidal activity of the toxins specifically exemplified herein. As used herein, the terms "variants" or "variations" of genes refer to nucleotide sequences which encode the same toxins or which encode equivalent toxins having pesticidal activity. As used herein, the term "equivalent toxins" refers to toxins having the same or essentially the same biological activity against the target pests as the claimed toxins.

It should be apparent to a person skilled in this art that genes encoding active toxins can be identified and obtained through several means. The specific genes exemplified herein may be obtained from the isolates deposited at a culture depository as described above. These genes, or portions or variants thereof, may also be constructed synthetically, for example, by use of a gene synthesizer. Variations of genes may be readily constructed using standard techniques for making point mutations. Also, fragments of these genes can be made using commercially available exonucleases or endonucleases according to standard procedures. For example, enzymes such as *Bal*31 or site-directed mutagenesis can be used to systematically cut off nucleotides from the ends of these genes. Also, genes which encode active fragments may be obtained using a variety of restriction enzymes. Proteases may be used to directly obtain active fragments of these toxins.

Equivalent toxins and/or genes encoding these equivalent toxins can be derived from *B.t.* isolates and/or DNA libraries using the teachings provided herein. There are a number of methods for obtaining the pesticidal toxins of the instant invention. For example, antibodies to the pesticidal toxins disclosed and claimed herein can be used to identify and isolate other toxins from a mixture of proteins. Specifically, antibodies may be raised to the portions of the toxins which are most constant and most distinct from other *B.t.* toxins. These antibodies can then be used to specifically identify equivalent toxins with the characteristic activity by immunoprecipitation, enzyme linked immunosorbent assay (ELISA), or western blotting. Antibodies to the toxins disclosed herein, or to equivalent toxins, or fragments of these toxins, can readily be prepared using standard procedures in this art. The genes which encode these toxins can then be obtained from the microorganism.

Fragments and equivalents which retain the pesticidal activity of the exemplified toxins would be within the scope of the subject invention. Also, because of the redundancy of the genetic code, a variety of different DNA sequences can encode the amino acid sequences disclosed herein. It is well within the skill of a person trained in the art to create these alternative DNA sequences encoding the same, or essentially the same, toxins. These variant DNA sequences are within the scope of the subject invention. As used herein, reference to "essentially the same" sequence refers to sequences which have amino acid substitutions, deletions, additions, or insertions which do not materially affect pesticidal activity. Fragments retaining pesticidal activity are also included in this definition.

A further method for identifying the toxins and genes of the subject invention is through the use of oligonucleotide probes. These probes are detectable nucleotide sequences. These sequences may be detectable by virtue of an appropriate label or may be made inherently

fluorescent as described in International Application No. WO93/16094. As is well known in the art, if the probe molecule and nucleic acid sample hybridize by forming a strong bond between the two molecules, it can be reasonably assumed that the probe and sample have substantial homology. Preferably, hybridization is conducted under stringent conditions by techniques well-known in the art, as described, for example, in Keller, G.H., M.M. Manak (1987) *DNA Probes*, Stockton Press, New York, NY., pp. 169-170. Detection of the probe provides a means for determining in a known manner whether hybridization has occurred. Such a probe analysis provides a rapid method for identifying toxin-encoding genes of the subject invention. The nucleotide segments which are used as probes according to the invention can be synthesized using a DNA synthesizer and standard procedures. These nucleotide sequences can also be used as PCR primers to amplify genes of the subject invention.

Certain toxins of the subject invention have been specifically exemplified herein. Since these toxins are merely exemplary of the toxins of the subject invention, it should be readily apparent that the subject invention comprises variant or equivalent toxins (and nucleotide sequences coding for equivalent toxins) having the same or similar pesticidal activity of the exemplified toxin. Equivalent toxins will have amino acid homology with an exemplified toxin. The amino acid identity will typically be greater than 60%, preferably be greater than 75%, more preferably greater than 80%, more preferably greater than 90%, and can be greater than 95%. The amino acid homology will be highest in critical regions of the toxin which account for biological activity or are involved in the determination of three-dimensional configuration which ultimately is responsible for the biological activity. In this regard, certain amino acid substitutions are acceptable and can be expected if these substitutions are in regions which are not critical to activity or are conservative amino acid substitutions which do not affect the three-dimensional configuration of the molecule. For example, amino acids may be placed in the following classes: non-polar, uncharged polar, basic, and acidic. Conservative substitutions whereby an amino acid of one class is replaced with another amino acid of the same type fall within the scope of the subject invention so long as the substitution does not materially alter the biological activity of the compound. Table 2 provides a listing of examples of amino acids belonging to each class.

Table 2.

Class of Amino Acid	Examples of Amino Acids
Nonpolar	Ala, Val, Leu, Ile, Pro, Met, Phe, Trp
Uncharged Polar	Gly, Ser, Thr, Cys, Tyr, Asn, Gln
Acidic	Asp, Glu
Basic	Lys, Arg, His

In some instances, non-conservative substitutions can also be made. The critical factor is that these substitutions must not significantly detract from the biological activity of the toxin.

The toxins of the subject invention can also be characterized in terms of the shape and location of toxin inclusions, which are described above.

Recombinant hosts. The toxin-encoding genes harbored by the isolates of the subject invention can be introduced into a wide variety of microbial or plant hosts. Expression of the toxin gene results, directly or indirectly, in the intracellular production and maintenance of the pesticide. With suitable microbial hosts, *e.g.*, *Pseudomonas*, the microbes can be applied to the situs of the pest, where they will proliferate and be ingested. The result is a control of the pest. Alternatively, the microbe hosting the toxin gene can be treated under conditions that prolong the activity of the toxin and stabilize the cell. The treated cell, which retains the toxic activity, then can be applied to the environment of the target pest.

Where the *B.t.* toxin gene is introduced via a suitable vector into a microbial host, and said host is applied to the environment in a living state, it is essential that certain host microbes be used. Microorganism hosts are selected which are known to occupy the "phytosphere" (phylloplane, phyllosphere, rhizosphere, and/or rhizoplane) of one or more crops of interest. These microorganisms are selected so as to be capable of successfully competing in the particular environment (crop and other insect habitats) with the wild-type microorganisms, provide for stable maintenance and expression of the gene expressing the polypeptide pesticide, and, desirably, provide for improved protection of the pesticide from environmental degradation and inactivation.

A large number of microorganisms are known to inhabit the phylloplane (the surface of the plant leaves) and/or the rhizosphere (the soil surrounding plant roots) of a wide variety of important crops. These microorganisms include bacteria, algae, and fungi. Of particular interest are microorganisms, such as bacteria, *e.g.*, genera *Pseudomonas*, *Erwinia*, *Serratia*, *Klebsiella*,

Xanthomonas, *Streptomyces*, *Rhizobium*, *Rhodopseudomonas*, *Methylophilius*, *Agrobacterium*, *Acetobacter*, *Lactobacillus*, *Arthrobacter*, *Azotobacter*, *Leuconostoc*, and *Alcaligenes*; fungi, particularly yeast, *e.g.*, genera *Saccharomyces*, *Cryptococcus*, *Kluyveromyces*, *Sporobolomyces*, *Rhodotorula*, and *Aureobasidium*. Of particular interest are such phytosphere bacterial species as *Pseudomonas syringae*, *Pseudomonas fluorescens*, *Serratia marcescens*, *Acetobacter xylinum*, *Agrobacterium tumefaciens*, *Rhodopseudomonas spheroides*, *Xanthomonas campestris*, *Rhizobium melioti*, *Alcaligenes entrophus*, and *Azotobacter vinlandii*; and phytosphere yeast species such as *Rhodotorula rubra*, *R. glutinis*, *R. marina*, *R. aurantiaca*, *Cryptococcus albidus*, *C. diffluens*, *C. laurentii*, *Saccharomyces rosei*, *S. pretoriensis*, *S. cerevisiae*, *Sporobolomyces roseus*, *S. odorus*, *Kluyveromyces veronae*, and *Aureobasidium pollulans*. Of particular interest are the pigmented microorganisms.

A wide variety of ways are available for introducing a *B.t.* gene encoding a toxin into a microorganism host under conditions which allow for stable maintenance and expression of the gene. These methods are well known to those skilled in the art and are described, for example, in United States Patent No. 5,135,867, which is incorporated herein by reference.

Control of coleopterans, including corn rootworm using the isolates, toxins, and genes of the subject invention can be accomplished by a variety of methods known to those skilled in the art. These methods include, for example, the application of *B.t.* isolates to the pests (or their location), the application of recombinant microbes to the pests (or their locations), and the transformation of plants with genes which encode the pesticidal toxins of the subject invention. Recombinant microbes may be, for example, a *B.t.*, *E. coli*, or *Pseudomonas*. Transformations can be made by those skilled in the art using standard techniques. Materials necessary for these transformations are disclosed herein or are otherwise readily available to the skilled artisan.

Synthetic genes which are functionally equivalent to the toxins of the subject invention can also be used to transform hosts. Methods for the production of synthetic genes can be found in, for example, U.S. Patent No. 5,380,831.

Control of other pests such as lepidopterans and other insects, nematodes, and mites can also be accomplished by those skilled in the art using standard techniques combined with the teachings provided herein.

Treatment of cells. As mentioned above, *B.t.* or recombinant cells expressing a *B.t.* toxin can be treated to prolong the toxin activity and stabilize the cell. The pesticide microcapsule that is formed comprises the *B.t.* toxin within a cellular structure that has been stabilized and will protect the toxin when the microcapsule is applied to the environment of the target pest. Suitable host cells may include either prokaryotes or eukaryotes, normally being

limited to those cells which do not produce substances toxic to higher organisms, such as mammals. However, organisms which produce substances toxic to higher organisms could be used, where the toxic substances are unstable or the level of application sufficiently low as to avoid any possibility of toxicity to a mammalian host. As hosts, of particular interest will be the prokaryotes and the lower eukaryotes, such as fungi.

The cell will usually be intact and be substantially in the proliferative form when treated, rather than in a spore form, although in some instances spores may be employed.

Treatment of the microbial cell, *e.g.*, a microbe containing the *B.t.* toxin gene, can be by chemical or physical means, or by a combination of chemical and/or physical means, so long as the technique does not deleteriously affect the properties of the toxin, nor diminish the cellular capability of protecting the toxin. Examples of chemical reagents are halogenating agents, particularly halogens of atomic no. 17-80. More particularly, iodine can be used under mild conditions and for sufficient time to achieve the desired results. Other suitable techniques include treatment with aldehydes, such as glutaraldehyde; anti-infectives, such as zephiran chloride and cetylpyridinium chloride; alcohols, such as isopropyl and ethanol; various histologic fixatives, such as Lugol iodine, Bouin's fixative, various acids and Helly's fixative (See: Humason, Gretchen L., *Animal Tissue Techniques*, W.H. Freeman and Company, 1967); or a combination of physical (heat) and chemical agents that preserve and prolong the activity of the toxin produced in the cell when the cell is administered to the host environment. Examples of physical means are short wavelength radiation such as gamma-radiation and X-radiation, freezing, UV irradiation, lyophilization, and the like. Methods for treatment of microbial cells are disclosed in United States Patent Nos. 4,695,455 and 4,695,462, which are incorporated herein by reference.

The cells generally will have enhanced structural stability which will enhance resistance to environmental conditions. Where the pesticide is in a proform, the method of cell treatment should be selected so as not to inhibit processing of the proform to the mature form of the pesticide by the target pest pathogen. For example, formaldehyde will crosslink proteins and could inhibit processing of the proform of a polypeptide pesticide. The method of treatment should retain at least a substantial portion of the bio-availability or bioactivity of the toxin.

Characteristics of particular interest in selecting a host cell for purposes of production include ease of introducing the *B.t.* gene into the host, availability of expression systems, efficiency of expression, stability of the pesticide in the host, and the presence of auxiliary genetic capabilities. Characteristics of interest for use as a pesticide microcapsule include protective qualities for the pesticide, such as thick cell walls, pigmentation, and intracellular

packaging or formation of inclusion bodies; survival in aqueous environments; lack of mammalian toxicity; attractiveness to pests for ingestion; ease of killing and fixing without damage to the toxin; and the like. Other considerations include ease of formulation and handling, economics, storage stability, and the like.

Growth of cells. The cellular host containing the *B.t.* insecticidal gene may be grown in any convenient nutrient medium, where the DNA construct provides a selective advantage, providing for a selective medium so that substantially all or all of the cells retain the *B.t.* gene. These cells may then be harvested in accordance with conventional ways. Alternatively, the cells can be treated prior to harvesting.

The *B.t.* cells of the invention can be cultured using standard art media and fermentation techniques. Upon completion of the fermentation cycle the bacteria can be harvested by first separating the *B.t.* spores and crystals from the fermentation broth by means well known in the art. The recovered *B.t.* spores and crystals can be formulated into a wettable powder, liquid concentrate, granules or other formulations by the addition of surfactants, dispersants, inert carriers, and other components to facilitate handling and application for particular target pests. These formulations and application procedures are all well known in the art.

Formulations. Formulated bait granules containing an attractant and spores and crystals of the *B.t.* isolates, or recombinant microbes comprising the genes obtainable from the *B.t.* isolates disclosed herein, can be applied to the soil. Formulated product can also be applied as a seed-coating or root treatment or total plant treatment at later stages of the crop cycle. Plant and soil treatments of *B.t.* cells may be employed as wettable powders, granules or dusts, by mixing with various inert materials, such as inorganic minerals (phyllosilicates, carbonates, sulfates, phosphates, and the like) or botanical materials (powdered corncobs, rice hulls, walnut shells, and the like). The formulations may include spreader-sticker adjuvants, stabilizing agents, other pesticidal additives, or surfactants. Liquid formulations may be aqueous-based or non-aqueous and employed as foams, gels, suspensions, emulsifiable concentrates, or the like. The ingredients may include rheological agents, surfactants, emulsifiers, dispersants, or polymers.

As would be appreciated by a person skilled in the art, the pesticidal concentration will vary widely depending upon the nature of the particular formulation, particularly whether it is a concentrate or to be used directly. The pesticide will be present in at least 1% by weight and may be 100% by weight. The dry formulations will have from about 1-95% by weight of the pesticide while the liquid formulations will generally be from about 1-60% by weight of the solids in the liquid phase. The formulations will generally have from about 10^2 to about 10^4

cells/mg. These formulations will be administered at about 50 mg (liquid or dry) to 1 kg or more per hectare.

The formulations can be applied to the environment of the pest, *e.g.*, soil and foliage, by spraying, dusting, sprinkling, or the like.

Mutants. Mutants of the isolates of the invention can be made by procedures well known in the art. For example, an asporogenous mutant can be obtained through ethylmethane sulfonate (EMS) mutagenesis of an isolate. The mutants can be made using ultraviolet light and nitrosoguanidine by procedures well known in the art.

A smaller percentage of the asporogenous mutants will remain intact and not lyse for extended fermentation periods; these strains are designated lysis minus (-). Lysis minus strains can be identified by screening asporogenous mutants in shake flask media and selecting those mutants that are still intact and contain toxin crystals at the end of the fermentation. Lysis minus strains are suitable for a cell treatment process that will yield a protected, encapsulated toxin protein.

To prepare a phage resistant variant of said asporogenous mutant, an aliquot of the phage lysate is spread onto nutrient agar and allowed to dry. An aliquot of the phage sensitive bacterial strain is then plated directly over the dried lysate and allowed to dry. The plates are incubated at 30°C. The plates are incubated for 2 days and, at that time, numerous colonies could be seen growing on the agar. Some of these colonies are picked and subcultured onto nutrient agar plates. These apparent resistant cultures are tested for resistance by cross streaking with the phage lysate. A line of the phage lysate is streaked on the plate and allowed to dry. The presumptive resistant cultures are then streaked across the phage line. Resistant bacterial cultures show no lysis anywhere in the streak across the phage line after overnight incubation at 30°C. The resistance to phage is then reconfirmed by plating a lawn of the resistant culture onto a nutrient agar plate. The sensitive strain is also plated in the same manner to serve as the positive control. After drying, a drop of the phage lysate is placed in the center of the plate and allowed to dry. Resistant cultures showed no lysis in the area where the phage lysate has been placed after incubation at 30°C for 24 hours.

Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1 – Culturing of *B.t.* Isolates of the Invention

A subculture of the *B.t.* isolates, or mutants thereof, can be used to inoculate the following medium, a peptone, glucose, salts medium.

Bacto Peptone	7.5 g/l
Glucose	1.0 g/l
KH_2PO_4	3.4 g/l
K_2HPO_4	4.35 g/l
Salt Solution	5.0 ml/l
$CaCl_2$ Solution	5.0 ml/l
pH 7.2	

Salts Solution (100 ml)

$MgSO_4 \cdot 7H_2O$	2.46 g
$MnSO_4 \cdot H_2O$	0.04 g
$ZnSO_4 \cdot 7H_2O$	0.28 g
$FeSO_4 \cdot 7H_2O$	0.40 g

$CaCl_2$ Solution (100 ml)

$CaCl_2 \cdot 2H_2O$	3.66 g

The salts solution and $CaCl_2$ solution are filter-sterilized and added to the autoclaved and cooked broth at the time of inoculation. Flasks are incubated at 30°C on a rotary shaker at 200 rpm for 64 hr.

The above procedure can be readily scaled up to large fermentors by procedures well known in the art.

The *B.t.* spores and/or crystals, obtained in the above fermentation, can be isolated by procedures well known in the art. A frequently-used procedure is to subject the harvested fermentation broth to separation techniques, *e.g.*, centrifugation.

Example 2 – Protein Purification for 45 kDa 80JJ1 Protein

One gram of lyophilized powder of 80JJ1 was suspended in 40 ml of buffer containing 80 mM Tris-Cl pH 7.8, 5 mM EDTA, 100 μM PMSF, 0.5 μg/ml Leupeptin, 0.7. μg/ml Pepstatin, and 40 μg/ml Bestatin. The suspension was centrifuged, and the resulting supernatant was

discarded. The pellet was washed five times using 35-40 ml of the above buffer for each wash. The washed pellet was resuspended in 10 ml of 40% NaBr, 5 mM EDTA, 100 μM PMSF, 0.5 μg/ml Leupeptin, 0.7 μg/ml Pepstatin, and 40 μg/ml Bestatin and placed on a rocker platform for 75 minutes. The NaBr suspension was centrifuged, the supernatant was removed, and the pellet was treated a second time with 40% NaBr, 5 mM EDTA, 100 μM PMSF, 0.5 μg/ml Leupeptin, 0.7 μg/ml Pepstatin, and 40 μg/ml Bestatin as above. The supernatants (40% NaBr soluble) were combined and dialyzed against 10 mM CAPS pH 10.0, 1 mM EDTA at 4°C. The dialyzed extracts were centrifuged and the resulting supernatant was removed. The pellet (40% NaBr dialysis pellet) was suspended in 5 ml of H_2O and centrifuged. The resultant supernatant was removed and discarded. The washed pellet was washed a second time in 10 ml of H_2O and centrifuged as above. The washed pellet was suspended in 1.5 ml of H_2O and contained primarily three peptides with molecular weights of approximately 47 kDa, 45 kDa, and 15 kDa when analyzed using SDS-PAGE. At this stage of purification, the suspended 40% NaBr dialysis pellet contained approximately 21 mg/ml of protein by Lowry assay.

The peptides in the pellet suspension were separated using SDS-PAGE (Laemlli, U.K. [1970] *Nature* 227:680) in 15% acrylamide gels. The separated proteins were then electrophoretically blotted to a PVDF membrane (Millipore Corp.) in 10 mM CAPS pH 11.0, 10% MeOH at 100 V constant. After one hour the PVDF membrane was rinsed in water briefly and placed for 3 minutes in 0.25% Coomassie blue R-250, 50% methanol, 5% acetic acid. The stained membrane was destained in 40% MeOH, 5% acetic acid. The destained membrane was air-dried at room temperature (LeGendre *et al.* [1989] In *A Practical Guide to Protein Purification For Microsequencing*, P. Matsudaira, ed., Academic Press, New York, NY). The membrane was sequenced using automated gas phase Edman degradation (Hunkapillar, M.W., R.M. Hewick, W.L. Dreyer, L.E. Hood [1983] *Meth. Enzymol.* 91:399).

The amino acid analysis revealed that the N-terminal sequence of the 45 kDa band was as follows: Met-Leu-Asp-Thr-Asn (SEQ ID NO. 1).

The 47 kDa band was also analyzed and the N-terminal amino acid sequence was determined to be the same 5-amino acid sequence as SEQ ID NO. 1. Therefore, the N-terminal amino acid sequences of the 47 kDa peptide and the 45 kDa peptide were identical.

This amino acid sequence also corresponds to a sequence obtained from a 45 kDa peptide obtained from PS80JJ1 spore/crystal powders, using another purification protocol, with the N-terminal sequence as follows: Met-Leu-Asp-Thr-Asn-Lys-Val-Tyr-Glu-Ile-Ser-Asn-Leu-Ala-Asn-Gly-Leu-Tyr-Thr-Ser-Thr-Tyr-Leu-Ser-Leu (SEQ ID NO. 2).

Example 3 – Purification of the 14 kDa Peptide of PS80JJ1

0.8 ml of the white dialysis suspension (approximately 21 mg/ml) containing the 47 kDa, 45 kDa, and 15 kDa peptides, was dissolved in 10 ml of 40% NaBr, and 0.5 ml of 100 mM EDTA were added. After about 18 hours (overnight), a white opaque suspension was obtained. This was collected by centrifugation and discarded. The supernatant was concentrated in a Centricon-30 (Amicon Corporation) to a final volume of approximately 15 ml. The filtered volume was washed with water by filter dialysis and incubated on ice, eventually forming a milky white suspension. The suspension was centrifuged and the pellet and supernatant were separated and retained. The pellet was then suspended in 1.0 ml water (approximately 6 mg/ml). The pellet contained substantially pure 15 kDa protein when analyzed by SDS-PAGE.

The N-terminal amino acid sequence was determined to be: Ser-Ala-Arg-Glu-Val-His-Ile-Glu-Ile-Asn-Asn-Thr-Arg-His-Thr-Leu-Gln-Leu-Glu-Ala-Lys-Thr-Lys-Leu (SEQ ID NO. 3).

Example 4 – Protein Purification and Characterization of PS149B1 45 kDa Protein

The P1 pellet was resuspended with two volumes of deionized water per unit wet weight, and to this was added nine volumes of 40% (w/w) aqueous sodium bromide. This and all subsequent operations were carried out on ice or at 4-6°C. After 30 minutes, the suspension was diluted with 36 volumes of chilled water and centrifuged at 25.000 x g for 30 minutes to give a pellet and a supernatant.

The resulting pellet was resuspended in 1-2 volumes of water and layered on a 20-40% (w/w) sodium bromide gradient and centrifuged at 8,000 x g for 100 minutes. The layer banding at approximately 32% (w/w) sodium bromide (the "inclusions", or INC) was recovered and dialyzed overnight against water using a dialysis membrane with a 6-8 kDa MW cut-off. Particulate material was recovered by centrifugation at 25,000 x g, resuspended in water, and aliquoted and assayed for protein by the method of Lowry and by SDS-PAGE.

The resulting supernatant was concentrated 3- to 4-fold using Centricon-10 concentrators, then dialyzed overnight against water using a dialysis membrane with a 6-8 kDa MW cut-off. Particulate material was recovered by centrifugation at 25,000 x g, resuspended in water, and aliquoted and assayed for protein by the method of Lowry and by SDS-PAGE. This fraction was denoted as P1.P2.

The peptides in the pellet suspension were separated using SDS-PAGE (Laemlli, U.K., *supra*) in 15% acrylamide gels. The separated proteins were then electrophoretically blotted to a PVDF membrane (Millipore Corp.) in 10 mM CAPS pH 11.0, 10% MeOH at 100 V constant.

After one hour the PVDF membrane was rinsed in water briefly and placed for 3 minutes in 0.25% Coomassie blue R-250, 50% methanol, 5% acetic acid. The stained membrane was destained in 40% MeOH, 5% acetic acid. The destained membrane was air-dried at room temperature (LeGendre *et al.*, *supra*). The membrane was sequenced using automated gas phase Edman degradation (Hunkapillar *et al.*, *supra*).

Protein analysis indicated the presence of two major polypeptides, with molecular weights of 47 kDa and 14 kDa. Molecular weights were measured against standard polypeptides of known molecular weight. This process provides only an estimate of true molecular weight. The 47 kDa band from PS149B1 migrated on SDS-PAGE in a manner indistinguishable from the 47 kDa protein from PS80JJ1. Likewise, the 14 kDa band from PS149B1 migrated on SDS-PAGE in a manner indistinguishable from 14 kDa bands from PS167H2 and PS80JJ1. Apart from these two polypeptides, which were estimated to account for 25-35% (47 kDa) and 35-55% (15 kDa) of the Coomassie staining material respectively, there may be minor bands, including those of estimated MW at 46 kDa, 130 kDa, and 70 kDa.

Protein analysis indicated that fraction INC contained a single polypeptide with MW of 47 kDa, and that fraction P1.P2 contained a single polypeptide with MW of 14 kDa. These polypeptides were recovered in yields greater than 50% from P1.

The N-terminal amino acid sequence for the purified 47 kDa protein from PS149B1 is: Met-Leu-Asp-Thr-Asn-Lys-Val-Tyr-Glu-Ile-Ser-Asn-His-Ala-Asn-Gly-Leu-Tyr-Ala-Ala-Thr-Tyr-Leu-Ser-Leu (SEQ ID NO. 4).

The N-terminal amino acid sequence for the purified 14 kDa protein from PS149B1 is: Ser-Ala-Arg-Glu-Val-His-Ile-Asp-Val-Asn-Asn-Lys-Thr-Gly-His-Thr-Leu-Gln-Leu-Glu-Asp-Lys-Thr-Lys-Leu-Asp-Gly-Gly-Arg-Trp-Arg-Thr-Ser-Pro-Xaa-Asn-Val-Ala-Asn-Asp-Gln-Ile-Lys-Thr-Phe-Val-Ala-Glu-Ser-Asn (SEQ ID NO. 5).

Example 5 – Amino Acid Sequence for 45 kDa and 14 kDa Toxins of PS167H2

The N-terminal amino acid sequence for the purified 45 kDa protein from PS167H2 is: Met-Leu-Asp-Thr-Asn-Lys-Ile-Tyr-Glu-Ile-Ser-Asn-Tyr-Ala-Asn-Gly-Leu-His-Ala-Ala-Thr-Tyr-Leu-Ser-Leu (SEQ ID NO. 6).

The N-terminal amino acid sequence for the purified 14 kDa protein from PS167H2 is: Ser-Ala-Arg-Glu-Val-His-Ile-Asp-Val-Asn-Asn-Lys-Thr-Gly-His-Thr-Leu-Gln-Leu-Glu-Asp-Lys-Thr-Lys-Leu (SEQ ID NO. 7).

These amino acid sequences can be compared to the sequence obtained for the 47 kDa peptide obtained from 80JJ1 spore/crystal powders with the N-terminal sequence (SEQ ID NO.

1) and to the sequence obtained for the 14 kDa peptide obtained from 80JJ1 spore/crystal powders with the N-terminal sequence (SEQ ID NO. 3).

Clearly, the 45-47 kDa proteins are highly related and probably represent one gene family, and the 14 kDa proteins are highly related and probably represent another gene family.

Example 6 – Molecular Cloning, Expression, and DNA Sequence Analysis of a Novel δ-Endotoxin Gene from *Bacillus thuringiensis* Strain PS80JJ1

Total cellular DNA was prepared from *Bacillus thuringiensis* (*B.t.*) cells grown to an optical density, at 600 nm, of 1.0. Cells were pelleted by centrifugation and resuspended in protoplast buffer (20 mg/ml lysozyme in 0.3 M sucrose, 25 mM Tris-Cl [pH 8.0], 25 mM EDTA). After incubation at 37°C for 1 hour, protoplasts were lysed by two cycles of freezing and thawing. Nine volumes of a solution of 0.1 M NaCl, 0.1% SDS, 0.1 M Tris-Cl were added to complete lysis. The cleared lysate was extracted twice with phenol:chloroform (1:1). Nucleic acids were precipitated with two volumes of ethanol and pelleted by centrifugation. The pellet was resuspended in TE buffer and RNase was added to a final concentration of 50 μg/ml. After incubation at 37°C for 1 hour, the solution was extracted once each with phenol:chloroform (1:1) and TE-saturated chloroform. DNA was precipitated from the aqueous phase by the addition of one-tenth volume of 3 M NaOAc and two volumes of ethanol. DNA was pelleted by centrifugation, washed with 70% ethanol, dried, and resuspended in TE buffer.

An oligonucleotide probe for the gene encoding the PS80JJ1 45 kDa toxin was designed from N-terminal peptide sequence data. The sequence of the 29-base oligonucleotide probe was:
5′-ATG YTW GAT ACW AAT AAA GTW TAT GAA AT-3′ (SEQ ID NO. 8)
This oligonucleotide was mixed at four positions as shown. This probe was radiolabeled with ^{32}P and used in standard condition hybridization of Southern blots of PS80JJ1 total cellular DNA digested with various restriction endonucleases. Representative autoradiographic data from these experiments showing the sizes of DNA restriction fragments containing sequence homology to the 44.3 kDa toxin oligonucleotide probe of SEQ ID NO. 8 are presented in Table 3.

Table 3. RFLP of PS80JJ1 cellular DNA fragments on Southern blots that hybridized under standard conditions with the 44.3 kDa toxin gene oligonucleotide probe (SEQ ID NO. 8)

Restriction Enzyme	Approximate Fragment Size (kbp)
*Eco*RI	6.0
*Hin*dIII	8.3
*Kpn*I	7.4
*Pst*I	11.5
*Xba*I	9.1

These DNA fragments identified in these analyses contain all or a segment of the PS80JJ1 45 kDa toxin gene. The approximate sizes of the hybridizing DNA fragments in Table 3 are in reasonable agreement with the sizes of a subset of the PS80JJ1 fragments hybridizing with a PS80JJ1 45 kDa toxin subgene probe used in separate experiments, as predicted (see Table 4, below).

A gene library was constructed from PS80JJ1 DNA partially digested with *Sau*3AI. Partial restriction digests were fractionated by agarose gel electrophoresis. DNA fragments 9.3 to 23 kbp in size were excised from the gel, electroeluted from the gel slice, purified on an Elutip-D ion exchange column (Schleicher and Schuell, Keene, NH), and recovered by ethanol precipitation. The *Sau*3AI inserts were ligated into *Bam*HI-digested LambdaGem-11 (Promega, Madison, WI). Recombinant phage were packaged and plated on *E. coli* KW251 cells. Plaques were screened by hybridization with the oligonucleotide probe described above. Hybridizing phage were plaque-purified and used to infect liquid cultures of *E. coli* KW251 cells for isolation of DNA by standard procedures (Maniatis *et al.*, *supra*).

Southern blot analysis revealed that one of the recombinant phage isolates contained an approximately 4.8 kbp *Xba*I-*Sac*I band that hybridized to the PS80JJ1 toxin gene probe. The *Sac*I site flanking the PS80JJ1 toxin gene is a phage vector cloning site, while the flanking *Xba*I site is located within the PS80JJ1 DNA insert. This DNA restriction fragment was subcloned by standard methods into pBluescript S/K (Stratagene, San Diego, CA) for sequence analysis. The resultant plasmid was designated pMYC2421. The DNA insert was also subcloned into pHTBluell (an *E. coli/B. thuringiensis* shuttle vector comprised of pBluescript S/K [Stratagene, La Jolla, CA] and the replication origin from a resident *B.t.* plasmid [D. Lereclus *et al.* (1989) *FEMS Microbiology Letters* 60:211-218]) to yield pMYC2420.

An oligonucleotide probe for the gene encoding the PS80JJ1 14 kDa toxin was designed from N-terminal peptide sequence data. The sequence of the 28-base oligonucleotide probe was: 5′ GW GAA GTW CAT ATW GAA ATW AAT AAT AC 3′ (SEQ ID NO. 29). This oligonucleotide was mixed at four positions as shown. The probe was radiolabelled with ^{32}P and used in standard condition hybridizations of Southern blots of PS80JJ1 total cellular and pMYC2421 DNA digested with various restriction endonucleases. These RFLP mapping experiments demonstrated that the gene encoding the 14 kDa toxin is located on the same genomic *EcoRI* fragment that contains the N-terminal coding sequence for the 44.3 kDa toxin.

To test expression of the PS80JJ1 toxin genes in *B.t.*, pMYC2420 was transformed into the acrystalliferous (Cry-) *B.t.* host, CryB (A. Aronson, Purdue University, West Lafayette, IN), by electroporation. Expression of both the approximately 14 and 44.3 kDa PS80JJ1 toxins encoded by pMYC2420 was demonstrated by SDS-PAGE analysis. Toxin crystal preparations from the recombinant CryB[pMYC2420] strain, MR536, were assayed and found to be active against western corn rootworm.

The PS80JJ1 toxin genes encoded by pMYC2421 were sequenced using the ABI373 automated sequencing system and associated software. The sequence of the entire genetic locus containing both open reading frames and flanking nucleotide sequences is shown in SEQ ID NO. 30. The termination codon of the 14 kDa toxin gene is 121 base pairs upstream (5′) from the initiation codon of the 44.3 kDa toxin gene (Figure 2). The PS80JJ1 14 kDa toxin open reading frame nucleotide sequence (SEQ ID NO. 31), the 44.3 kDa toxin open reading frame nucleotide sequence (SEQ ID NO. 10), and the respective deduced amino acid sequences (SEQ ID NO. 32 and SEQ ID NO. 11) are novel compared to other toxin genes encoding pesticidal proteins.

Thus, the nucleotide sequence encoding the 14 kDa toxin of PS80JJ1 is shown in SEQ ID NO. 31. The deduced amino acid sequence of the 14 kDa toxin of PS80JJ1 is shown in SEQ ID NO. 32. The nucleotide sequences encoding both the 14 and 45 kDa toxins of PS80JJ1, as well as the flanking sequences, are shown in SEQ ID NO. 30. The relationship of these sequences is shown in Figure 2.

A subculture of *E. coli* NM522 containing plasmid pMYC2421 was deposited in the permanent collection of the Patent Culture Collection (NRRL), Regional Research Center, 1815 North University Street, Peoria, IL 61604 USA on March 28, 1996. The accession number is NRRL B-21555.

Example 7 – RFLP and PCR Analysis of Additional Novel δ-Endotoxin Genes from *Bacillus thuringiensis* Strains PS149B1 and PS167H2

Two additional strains active against corn rootworm, PS149B1 and PS167H2, also produce parasporal protein crystals comprised in part of polypeptides approximately 14 and 45 kDa in size. Southern hybridization and partial DNA sequence analysis were used to examine the relatedness of these toxins to the 80JJ1 toxins. DNA was extracted from these *B.t.* strains as described above, and standard Southern hybridizations were performed using the 14 kDa toxin oligonucleotide probe (SEQ ID NO. 29) and an approximately 800 bp PCR fragment of the 80JJ1 44.3 kDa toxin gene-encoding sequence. Representative RFLP data from these experiments showing the sizes of DNA restriction fragments containing sequence homology to the 44.3 kDa toxin are presented in Table 4. Representative RFLP data from these experiments showing the sizes of DNA restriction fragments containing sequence homology to the approximately 14 kDa toxin are presented in Table 5.

Table 4. RFLP of PS80JJ1, PS149B1, and PS167H2 cellular DNA fragments on Southern blots that hybridized with the approximately 800 bp PS80JJ1 44.3 kDa toxin subgene probe under standard conditions

	Strain		
	PS80JJ1	PS149B1	PS167H2
Restriction enzyme	Approximate fragment size (kbp)		
*Eco*RI	6.4	5.7	2.6
	1.3	2.8	
	0.6		
*Hin*dIII	8.2	6.2	4.4
*Kpn*I	7.8	10.0	11.5
*Pst*I	12.0	9.2	9.2
			8.2
*Xba*I	9.4	10.9	10.9
*Sac*I	17.5	15.5	11.1
	13.1	10.5	6.3

Each of the three strains exhibited unique RFLP patterns. The hybridizing DNA fragments from PS149B1 or PS167H2 contain all or part of toxin genes with sequence homology to the PS80JJ1 44.3 kDa toxin.

Table 5. Restriction fragment length polymorphisms of PS80JJ1, PS149B1, and PS167H2 cellular DNA fragments on Southern blots that hybridized with the PS80JJ1 14 kDa toxin oligonucleotide probe under standard conditions

	Strain		
	PS80JJ1	PS149B1	PS167H2
Restriction enzyme	Approximate fragment size (kbp)		
*Eco*RI	5.6	2.7	2.7
HindIII	7.1	6.0	4.7
*Xba*I	8.4	11.2	11.2

Each of the three strains exhibited unique RFLP patterns. The hybridizing DNA fragments from PS149B1 or PS167H2 contain all or part of toxin genes with sequence homology to the PS80JJ1 14 kDa toxin gene.

Portions of the toxin genes in PS149B1 or PS167H2 were amplified by PCR using forward and reverse oligonucleotide primer pairs designed based on the PS80JJ1 44.3 kDa toxin gene sequence. For PS149B1, the following primer pair was used:

Forward:

5'-ATG YTW GAT ACW AAT AAA GTW TAT GAA AT-3' (SEQ ID NO. 8)

Reverse:

5'-GGA TTA TCT ATC TCT GAG TGT TCT TG-3' (SEQ ID NO. 9)

For PS167H2, the same primer pair was used. These PCR-derived fragments were sequenced using the ABI373 automated sequencing system and associated software. The partial gene and peptide sequences obtained are shown in SEQ ID NO. 12-15. These sequences contain portions of the nucleotide coding sequences and peptide sequences for novel corn rootworm-active toxins present in *B.t.* strains PS149B1 or PS167H2.

Example 8 – Molecular Cloning and DNA Sequence Analysis of Novel δ-Endotoxin Genes from *Bacillus thuringiensis* Strains PS149B1 and PS167H2

Total cellular DNA was extracted from strains PS149B1 and PS167H2 as described for PS80JJ1. Gene libraries of size-fractionated *Sau*3A partial restriction fragments were constructed in Lambda-Gem11 for each respective strain as previously described. Recombinant phage were packaged and plated on *E. coli* KW251 cells. Plaques were screened by hybridization with the oligonucleotide probe specific for the 44 kDa toxin gene. Hybridizing phage were plaque-purified and used to infect liquid cultures of *E. coli* KW251 cells for isolation of DNA by standard procedures (Maniatis *et al., supra*).

For PS167H2, Southern blot analysis revealed that one of the recombinant phage isolates contained an approximately 4.0 to 4.4 kbp *Hin*dIII band that hybridized to the PS80JJ1 44 kDa toxin gene 5′ oligonucleotide probe (SEQ ID NO. 8). This DNA restriction fragment was subcloned by standard methods into pBluescript S/K (Stratgene, San Diego, CA) for sequence analysis. The fragment was also subcloned into the high copy number shuttle vector, pHT370 (Arantes, O., D. Lereclus [1991] *Gene* 108:115-119) for expression analyses in *Bacillus thuringiensis* (see below). The resultant recombinant, high copy number bifunctional plasmid was designated pMYC2427.

The PS167H2 toxin genes encoded by pMYC2427 were sequenced using the ABI automated sequencing system and associated software. The sequence of the entire genetic locus containing both open reading frames and flanking nucleotide sequences is shown in SEQ ID NO. 34. The termination codon of the 14 kDa toxin gene is 107 base pairs upstream (5′) from the initiation codon of the 44 kDa toxin gene. The PS167H2 14 kDa toxin coding sequence (SEQ ID NO. 35), the 44 kDa toxin coding sequence (SEQ ID NO. 37), and the respective deduced amino acid sequences, SEQ ID NO. 36 and SEQ ID NO. 38, are novel compared to other known toxin genes encoding pesticidal proteins. The toxin genes are arranged in a similar manner to, and have some homology with, the PS80JJ1 14 and 44 kDa toxins.

A subculture of *E. coli* NM522 containing plasmid pMYC2427 was deposited in the permanent collection of the Patent Culture Collection (NRRL), Regional Research Center, 1815 North University Street, Peoria, Illinois 61604 USA on 26 March 1997. The accession number is NRRL B-21672.

For PS149B1, Southern blot analysis using the PS80JJ1 44 kDa oligonucleotide 5′ probe (SEQ ID NO. 8) demonstrated hybridization of an approximately 5.9 kbp *Cla*I DNA fragment. Complete *Cla*I digests of PS149B1 genomic DNA were size fractionated on agarose gels and cloned into pHTBlueII. The fragment was also subcloned into the high copy number shuttle

vector, pHT370 (Arantes, O., D. Lereclus [1991] *Gene* 108:115-119) for expression analyses in *Bacillus thuringiensis* (see below). The resultant recombinant, high copy number bifunctional plasmid was designated pMYC2429.

The PS149B1 toxin genes encoded by pMYC2429 were sequenced using the ABI automated sequencing system and associated software. The sequence of the entire genetic locus containing both open reading frames and flanking nucleotide sequences is shown in SEQ ID NO. 39. The termination codon of the 14 kDa toxin gene is 108 base pairs upstream (5') from the initiation codon of the 44 kDa toxin gene. The PS149B1 14 kDa toxin coding sequence (SEQ ID NO. 40), the 44 kDa toxin coding sequence (SEQ ID NO. 42), and the respective deduced amino acid sequences, SEQ ID NO. 41 and SEQ ID NO. 43, are novel compared to other known toxin genes encoding pesticidal proteins. The toxin genes are arranged in a similar manner as, and have some homology with, the PS80JJ1 and PS167H2 14 and 44 kDa toxins. Together, these three toxin operons comprise a new family of pesticidal toxins.

A subculture of *E. coli* NM522 containing plasmid pMYC2429 was deposited in the permanent collection of the Patent Culture Collection (NRRL), Regional Research Center, 1815 North University Street, Peoria, Illinois 61604 USA on 26 March 1997. The accession number is NRRL B-21673.

Example 9 – PCR Amplification for Identification and Cloning Novel Corn Rootworm-Active Toxin

The DNA and peptide sequences of the three novel approximately 45 kDa corn rootworm-active toxins from PS80JJ1, PS149B1, and PS167H2 (SEQ ID NOS. 12-15) were aligned with the Genetics Computer Group sequence analysis program Pileup using a gap weight of 3.00 and a gap length weight of 0.10. The sequence alignments were used to identify conserved peptide sequences to which oligonucleotide primers were designed that were likely to hybridize to genes encoding members of this novel toxin family. Such primers can be used in PCR to amplify diagnostic DNA fragments for these and related toxin genes. Numerous primer designs to various sequences are possible, four of which are described here to provide an example. These peptide sequences are:

Asp-Ile-Asp-Asp-Tyr-Asn-Leu (SEQ ID NO. 16)

Trp-Phe-Leu-Phe-Pro-Ile-Asp (SEQ ID NO. 17)

Gln-Ile-Lys-Thr-Thr-Pro-Tyr-Tyr (SEQ ID NO. 18)

Tyr-Glu-Trp-Gly-Thr-Glu (SEQ ID NO. 19).

The corresponding nucleotide sequences are:

5′-GATATWGATGAYTAYAAYTTR-3′ (SEQ ID NO. 20)

5′-TGGTTTTTRTTTCCWATWGAY-3′ (SEQ ID NO. 21)

5′-CAAATHAAAACWACWCCATATTAT-3′ (SEQ ID NO. 22)

5′-TAYGARTGGGGHACAGAA-3′ (SEQ ID NO. 23).

Forward primers for polymerase amplification in thermocycle reactions were designed based on the nucleotide sequences of SEQ ID NOS. 20 and 21.

Reverse primers were designed based on the reverse complement of SEQ ID NOS. 22 and 23:

5′-ATAATATGGWGTWGTTTTDATTTG-3′ (SEQ ID NO. 24)

5′-TTCTGTDCCCCAYTCRTA-3′ (SEQ ID NO. 25).

These primers can be used in combination to amplify DNA fragments of the following sizes (Table 6) that identify genes encoding novel corn rootworm toxins.

Table 6. Predicted sizes of diagnostic DNA fragments (base pairs) amplifiable with primers specific for novel corn rootworm-active toxins

Primer pair (SEQ ID NO.)	DNA fragment size (bp)
20 + 24	495
20 + 25	594
21 + 24	471
21 + 25	580

Similarly, entire genes encoding novel corn rootworm-active toxins can be isolated by polymerase amplification in thermocycle reactions using primers designed based on DNA sequences flanking the open reading frames. For the PS80JJ1 44.3 kDa toxin, one such primer pair was designed, synthesized, and used to amplify a diagnostic 1613 bp DNA fragment that included the entire toxin coding sequence. These primers are:

Forward: 5′-CTCAAAGCGGATCAGGAG-3′ (SEQ ID NO. 26)

Reverse: 5′-GCGTATTCGGATATGCTTGG-3′ (SEQ ID NO. 27).

For PCR amplification of the PS80JJ1 14 kDa toxin, the oligonucleotide coding for the N-terminal peptide sequence (SEQ ID NO. 29) can be used in combination with various reverse oligonucleotide primers based on the sequences in the PS80JJ1 toxin gene locus. One such reverse primer has the following sequence:

5′ CATGAGATTTATCTCCTGATCCGC 3′ (SEQ ID NO. 33).

When used in standard PCR reactions, this primer pair amplified a diagnostic 1390 bp DNA fragment that includes the entire 14 kDa toxin coding sequence and some 3′ flanking sequences corresponding to the 121 base intergenic spacer and a portion of the 44.3 kDa toxin gene. When used in combination with the 14 kDa forward primer, PCR will generate a diagnostic 322 base pair DNA fragment.

Example 10 – Bioassay of Protein

A preparation of the insoluble fraction from the dialyzed NaBr extract of 80JJ1 containing the 47 kDa, 45 kDa, and 15 kDa peptides was bioassayed against Western corn rootworm and found to exhibit significant toxin activity.

Example 11 – Bioassay of Protein

The purified protein fractions from PS149B1 were bioassayed against western corn rootworm and found to exhibit significant toxin activity when combined. In fact, the combination restored activity to that noted in the original preparation (P1). The following bioassay data set presents percent mortality and demonstrates this effect.

Table 7.

Concentration (μg/cm²)	P1	INC	P1.P2	INC + P1.P2
300	88, 100, 94	19	13	100
100	94, 50, 63	31	38	94
33.3	19, 19, 44	38	13	50
11.1	13, 56, 25	12	31	13
3.7	0, 50, 0	0	31	13
1.2	13, 43, 12	0	12	19
0.4	6, 12, 6	25	19	6

Example 12 – Clone Dose-Response Bioassays

The PS80JJ1 toxin operon was also subcloned from pMYC2421 into pHT370 for direct comparison of bioactivity with the recombinant toxins cloned from PS149B1 and PS167H2. The resultant recombinant, high copy number bifunctional plasmid was designated pMYC2426.

A subculture of *E. coli* NM522 containing plasmid pMYC2426 was deposited in the permanent collection of the Patent Culture Collection (NRRL), Regional Research Center, 1815 North University Street, Peoria, Illinois 61604 USA on 26 March 1997. The accession number is NRRL B-21671.

To test expression of the PS80JJ1, PS149B1 and PS167H2 toxin genes in *B.t.*, pMYC2426, pMYC2427 and pMYC2429 were separately transformed into the acrystalliferous (Cry-) *B. t.* host, CryB (A. Aronson, Purdue University, West Lafayette, IN), by electroporation. The recombinant strains were designated MR543 (CryB [pMYC2426]), MR544 (CryB [pMYC2427]) and MR546 (CryB [pMYC2429]), respectively . Expression of both the approximately 14 and 44 kDa toxins was demonstrated by SDS-PAGE analysis for each recombinant strain.

Toxin crystal preparations from the recombinant strains were assayed against western corn rootworm. Their diet was amended with sorbic acid and SIGMA pen-strep-ampho-B. The material was top-loaded at a rate of 50 µl of suspension per cm^2 diet surface area. Bioassays were run with neonate Western corn rootworm larvae for 4 days at approximately 25°C. Percentage mortality and top-load LC_{50} estimates for the clones (pellets) are set forth in Table 8.

Table 8.

	Percentage mortality at given protein concentration (µg/cm^2)		
Sample	50	5	0.5
MR543 pellet	44	19	9
MR544 pellet	72	32	21
MR546 pellet	52	32	21
dH2O	7		

Example 13 – Insertion and Expression of Toxin Genes Into Plants

One aspect of the subject invention is the transformation of plants with genes encoding the insecticidal toxin. The transformed plants are resistant to attack by the target pest.

The novel corn rootworm-active genes described here can be optimized for expression in other organisms. Maize optimized gene sequences encoding the 14 and 44 kDa PS80JJ1 toxins are disclosed in SEQ ID NO. 44 and SEQ ID NO. 45, respectively.

Genes encoding pesticidal toxins, as disclosed herein, can be inserted into plant cells using a variety of techniques which are well known in the art. For example, a large number of cloning vectors comprising a replication system in *E. coli* and a marker that permits selection of the transformed cells are available for preparation for the insertion of foreign genes into higher plants. The vectors comprise, for example, pBR322, pUC series, M13mp series, pACYC184, etc. Accordingly, the sequence encoding the *B.t.* toxin can be inserted into the vector at a suitable restriction site. The resulting plasmid is used for transformation into *E. coli*. The *E. coli* cells are cultivated in a suitable nutrient medium, then harvested and lysed. The plasmid is recovered. Sequence analysis, restriction analysis, electrophoresis, and other biochemical-molecular biological methods are generally carried out as methods of analysis. After each manipulation, the DNA sequence used can be cleaved and joined to the next DNA sequence. Each plasmid sequence can be cloned in the same or other plasmids. Depending on the method of inserting desired genes into the plant, other DNA sequences may be necessary. If, for example, the Ti or Ri plasmid is used for the transformation of the plant cell, then at least the right border, but often the right and the left border of the Ti or Ri plasmid T-DNA, has to be joined as the flanking region of the genes to be inserted.

The use of T-DNA for the transformation of plant cells has been intensively researched and sufficiently described in EP 120 516; Hoekema (1985) In: *The Binary Plant Vector System*, Offset-durkkerij Kanters B.V., Alblasserdam, Chapter 5; Fraley *et al.*, *Crit. Rev. Plant Sci.* 4:1-46; and An *et al.* (1985) *EMBO J.* 4:277-287.

Once the inserted DNA has been integrated in the genome, it is relatively stable there and, as a rule, does not come out again. It normally contains a selection marker that confers on the transformed plant cells resistance to a biocide or an antibiotic, such as kanamycin, G 418, bleomycin, hygromycin, or chloramphenicol, *inter alia*. The individually employed marker should accordingly permit the selection of transformed cells rather than cells that do not contain the inserted DNA.

A large number of techniques are available for inserting DNA into a plant host cell. Those techniques include transformation with T-DNA using *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* as transformation agent, fusion, injection, biolistics (microparticle bombardment), or electroporation as well as other possible methods. If Agrobacteria are used for the transformation, the DNA to be inserted has to be cloned into special plasmids, namely either into an intermediate vector or into a binary vector. The intermediate vectors can be integrated into the Ti or Ri plasmid by homologous recombination owing to sequences that are homologous to sequences in the T-DNA. The Ti or Ri plasmid also comprises the vir region

necessary for the transfer of the T-DNA. Intermediate vectors cannot replicate themselves in Agrobacteria. The intermediate vector can be transferred into *Agrobacterium tumefaciens* by means of a helper plasmid (conjugation). Binary vectors can replicate themselves both in *E. coli* and in Agrobacteria. They comprise a selection marker gene and a linker or polylinker which are framed by the right and left T-DNA border regions. They can be transformed directly into Agrobacteria (Holsters *et al.* [1978] *Mol. Gen. Genet.* 163:181-187). The *Agrobacterium* used as host cell is to comprise a plasmid carrying a vir region. The vir region is necessary for the transfer of the T-DNA into the plant cell. Additional T-DNA may be contained. The bacterium so transformed is used for the transformation of plant cells. Plant explants can advantageously be cultivated with *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* for the transfer of the DNA into the plant cell. Whole plants can then be regenerated from the infected plant material (for example, pieces of leaf, segments of stalk, roots, but also protoplasts or suspension-cultivated cells) in a suitable medium, which may contain antibiotics or biocides for selection. The plants so obtained can then be tested for the presence of the inserted DNA. No special demands are made of the plasmids in the case of injection and electroporation. It is possible to use ordinary plasmids, such as, for example, pUC derivatives.

The transformed cells grow inside the plants in the usual manner. They can form germ cells and transmit the transformed trait(s) to progeny plants. Such plants can be grown in the normal manner and crossed with plants that have the same transformed hereditary factors or other hereditary factors. The resulting hybrid individuals have the corresponding phenotypic properties.

In a preferred embodiment of the subject invention, plants will be transformed with genes wherein the codon usage has been optimized for plants. See, for example, U.S. Patent No. 5,380,831, which is hereby incorporated by reference. Also, advantageously, plants encoding a truncated toxin will be used. The truncated toxin typically will encode about 55% to about 80% of the full length toxin. Methods for creating synthetic *B.t.* genes for use in plants are known in the art.

Example 14 – Cloning of *B.t.* Genes Into Insect Viruses

A number of viruses are known to infect insects. These viruses include, for example, baculoviruses and entomopoxviruses. In one embodiment of the subject invention, genes encoding the insecticidal toxins, as described herein, can be placed within the genome of the insect virus, thus enhancing the pathogenicity of the virus. Methods for constructing insect viruses which comprise *B.t.* toxin genes are well known and readily practiced by those skilled

in the art. These procedures are described, for example, in Merryweather *et al.* (Merryweather, A.T., U. Weyer, M.P.G. Harris, M. Hirst, T. Booth, R.D. Possee (1990) *J. Gen. Virol. 71*:1535-1544) and Martens *et al.* (Martens, J.W.M., G. Honee, D. Zuidema, J.W.M. van Lent, B. Visser, J.M. Vlak (1990) *Appl. Environmental Microbiol. 56*(9):2764-2770).

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It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

SEQUENCE LISTING

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/633,993
(B) FILING DATE: 19-APR-1996
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Leu Asp Thr Asn
1 5

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Leu Asp Thr Asn Lys Val Tyr Glu Ile Ser Asn Leu Ala Asn Gly
1 5 10 15

Leu Tyr Thr Ser Thr Tyr Leu Ser Leu
20 25

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ser Ala Arg Glu Val His Ile Glu Ile Asn Asn Thr Arg His Thr Leu
1 5 10 15

Gln Leu Glu Ala Lys Thr Lys Leu
20

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Leu Asp Thr Asn Lys Val Tyr Glu Ile Ser Asn His Ala Asn Gly
1 5 10 15

Leu Tyr Ala Ala Thr Tyr Leu Ser Leu
20 25

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ser Ala Arg Glu Val His Ile Asp Val Asn Asn Lys Thr Gly His Thr
1 5 10 15

Leu Gln Leu Glu Asp Lys Thr Lys Leu Asp Gly Gly Arg Trp Arg Thr
20 25 30

Ser Pro Xaa Asn Val Ala Asn Asp Gln Ile Lys Thr Phe Val Ala Glu
35 40 45

Ser Asn
50

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Leu Asp Thr Asn Lys Ile Tyr Glu Ile Ser Asn Tyr Ala Asn Gly
1 5 10 15

Leu His Ala Ala Thr Tyr Leu Ser Leu
20 25

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ser Ala Arg Glu Val His Ile Asp Val Asn Asn Lys Thr Gly His Thr
1 5 10 15

Leu Gln Leu Glu Asp Lys Thr Lys Leu
20 25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATGNTNGATA CNAATAAAGT NTATGAAAT 29

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGATTATCTA TCTCTGAGTG TTCTTG 26

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1158 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ATGTTAGATA CTAATAAAGT TTATGAAATA AGCAATCTTG CTAATGGATT ATATACATCA 60

ACTTATTTAA GTCTTGATGA TTCAGGTGTT AGTTTAATGA GTAAAAAGGA TGAAGATATT 120

GATGATTACA ATTTAAAATG GTTTTTATTT CCTATTGATA ATAATCAATA TATTATTACA 180

AGCTATGGAG CTAATAATTG TAAAGTTTGG AATGTTAAAA ATGATAAAAT AAATGTTTCA 240

ACTTATTCTT CAACAAACTC TGTACAAAAA TGGCAAATAA AAGCTAAAGA TTCTTCATAT 300

ATAATACAAA GTGATAATGG AAAGGTCTTA ACAGCAGGAG TAGGTCAATC TCTTGGAATA 360

GTACGCCTAA CTGATGAATT TCCAGAGAAT TCTAACCAAC AATGGAATTT AACTCCTGTA 420

CAAACAATTC AACTCCCACA AAAACCTAAA ATAGATGAAA AATTAAAAGA TCATCCTGAA 480

TATTCAGAAA CCGGAAATAT AAATCCTAAA ACAACTCCTC AATTAATGGG ATGGACATTA 540

GTACCTTGTA TTATGGTAAA TGATTCAAAA ATAGATAAAA ACACTCAAAT TAAAACTACT 600

CCATATTATA TTTTTAAAAA ATATAAATAC TGGAATCTAG CAAAAGGAAG TAATGTATCT 660

TTACTTCCAC ATCAAAAAAG ATCATATGAT TATGAATGGG GTACAGAAAA AAATCAAAAA 720

ACAACTATTA TTAATACAGT AGGATTGCAA ATTAATATAG ATTCAGGAAT GAAATTTGAA 780

GTACCAGAAG TAGGAGGAGG TACAGAAGAC ATAAAAACAC AATTAACTGA AGAATTAAAA 840

GTTGAATATA GCACTGAAAC CAAAATAATG ACGAAATATC AAGAACACTC AGAGATAGAT 900

AATCCAACTA ATCAACCAAT GAATTCTATA GGACTTCTTA TTTATACTTC TTTAGAATTA 960

TATCGATATA ACGGTACAGA AATTAAGATA ATGGACATAG AAACTTCAGA TCATGATACT 1020

TACACTCTTA CTTCTTATCC AAATCATAAA GAAGCATTAT TACTTCTCAC AAACCATTCG 1080

TATGAAGAAG TAGAAGAAAT AACAAAAATA CCTAAGCATA CACTTATAAA ATTGAAAAAA 1140

CATTATTTTA AAAAATAA 1158

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 385 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Leu Asp Thr Asn Lys Val Tyr Glu Ile Ser Asn Leu Ala Asn Gly
1 5 10 15

```
Leu Tyr Thr Ser Thr Tyr Leu Ser Leu Asp Asp Ser Gly Val Ser Leu
            20                  25                  30

Met Ser Lys Lys Asp Glu Asp Ile Asp Asp Tyr Asn Leu Lys Trp Phe
        35                  40                  45

Leu Phe Pro Ile Asp Asn Asn Gln Tyr Ile Ile Thr Ser Tyr Gly Ala
    50                  55                  60

Asn Asn Cys Lys Val Trp Asn Val Lys Asn Asp Lys Ile Asn Val Ser
65                  70                  75                  80

Thr Tyr Ser Ser Thr Asn Ser Val Gln Lys Trp Gln Ile Lys Ala Lys
                85                  90                  95

Asp Ser Ser Tyr Ile Ile Gln Ser Asp Asn Gly Lys Val Leu Thr Ala
            100                 105                 110

Gly Val Gly Gln Ser Leu Gly Ile Val Arg Leu Thr Asp Glu Phe Pro
        115                 120                 125

Glu Asn Ser Asn Gln Gln Trp Asn Leu Thr Pro Val Gln Thr Ile Gln
    130                 135                 140

Leu Pro Gln Lys Pro Lys Ile Asp Glu Lys Leu Lys Asp His Pro Glu
145                 150                 155                 160

Tyr Ser Glu Thr Gly Asn Ile Asn Pro Lys Thr Thr Pro Gln Leu Met
                165                 170                 175

Gly Trp Thr Leu Val Pro Cys Ile Met Val Asn Asp Ser Lys Ile Asp
            180                 185                 190

Lys Asn Thr Gln Ile Lys Thr Thr Pro Tyr Tyr Ile Phe Lys Lys Tyr
        195                 200                 205

Lys Tyr Trp Asn Leu Ala Lys Gly Ser Asn Val Ser Leu Leu Pro His
    210                 215                 220

Gln Lys Arg Ser Tyr Asp Tyr Glu Trp Gly Thr Glu Lys Asn Gln Lys
225                 230                 235                 240

Thr Thr Ile Ile Asn Thr Val Gly Leu Gln Ile Asn Ile Asp Ser Gly
                245                 250                 255

Met Lys Phe Glu Val Pro Glu Val Gly Gly Gly Thr Glu Asp Ile Lys
            260                 265                 270

Thr Gln Leu Thr Glu Glu Leu Lys Val Glu Tyr Ser Thr Glu Thr Lys
        275                 280                 285

Ile Met Thr Lys Tyr Gln Glu His Ser Glu Ile Asp Asn Pro Thr Asn
    290                 295                 300

Gln Pro Met Asn Ser Ile Gly Leu Leu Ile Tyr Thr Ser Leu Glu Leu
305                 310                 315                 320
```

Tyr Arg Tyr Asn Gly Thr Glu Ile Lys Ile Met Asp Ile Glu Thr Ser
325 330 335

Asp His Asp Thr Tyr Thr Leu Thr Ser Tyr Pro Asn His Lys Glu Ala
340 345 350

Leu Leu Leu Leu Thr Asn His Ser Tyr Glu Glu Val Glu Glu Ile Thr
355 360 365

Lys Ile Pro Lys His Thr Leu Ile Lys Leu Lys Lys His Tyr Phe Lys
370 375 380

Lys
385

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 834 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGACTATATG CAGCAACTTA TTTAAGTTTA GATGATTCAG GTGTTAGTTT AATGAATAAA 60

AATGATGATG ATATTGATGA TTATAACTTA AAATGGTTTT TATTTCCTAT TGATGATGAT 120

CAATATATTA TTACAAGCTA TGCAGCAAAT AATTGTAAAG TTTGGAATGT TAATAATGAT 180

AAAATAAATG TTTCGACTTA TTCTTCAACA AATTCAATAC AAAAATGGCA AATAAAAGCT 240

AATGGTTCTT CATATGTAAT ACAAAGTGAT AATGGAAAAG TCTTAACAGC AGGAACCGGT 300

CAAGCTCTTG GATTGATACG TTTAACTGAT GAATCCTCAA ATAATCCCAA TCAACAATGG 360

AATTTAACTT CTGTACAAAC AATTCAACTT CCACAAAAAC CTATAATAGA TACAAAATTA 420

AAAGATTATC CCAAATATTC ACCAACTGGA AATATAGATA ATGGAACATC TCCTCAATTA 480

ATGGGATGGA CATTAGTACC TTGTATTATG GTAAATGATC CAAATATAGA TAAAAATACT 540

CAAATTAAAA CTACTCCATA TTATATTTTA AAAAAATATC AATATTGGCA ACGAGCAGTA 600

GGAAGTAATG TAGCTTTACG TCCACATGAA AAAAAATCAT ATACTTATGA ATGGGGCACA 660

GAAATAGATC AAAAAACAAC AATTATAAAT ACATTAGGAT TTCAAATCAA TATAGATTCA 720

GGAATGAAAT TTGATATACC AGAAGTAGGT GGAGGTACAG ATGAAATAAA AACACAACTA 780

AATGAAGAAT TAAAAATAGA ATATAGTCAT GAAACTAAAA TAATGGAAAA ATAT 834

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 278 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Gly Leu Tyr Ala Ala Thr Tyr Leu Ser Leu Asp Asp Ser Gly Val Ser
1               5                  10                  15

Leu Met Asn Lys Asn Asp Asp Asp Ile Asp Asp Tyr Asn Leu Lys Trp
            20                  25                  30

Phe Leu Phe Pro Ile Asp Asp Asp Gln Tyr Ile Ile Thr Ser Tyr Ala
        35                  40                  45

Ala Asn Asn Cys Lys Val Trp Asn Val Asn Asn Asp Lys Ile Asn Val
    50                  55                  60

Ser Thr Tyr Ser Ser Thr Asn Ser Ile Gln Lys Trp Gln Ile Lys Ala
65                  70                  75                  80

Asn Gly Ser Ser Tyr Val Ile Gln Ser Asp Asn Gly Lys Val Leu Thr
                85                  90                  95

Ala Gly Thr Gly Gln Ala Leu Gly Leu Ile Arg Leu Thr Asp Glu Ser
            100                 105                 110

Ser Asn Asn Pro Asn Gln Gln Trp Asn Leu Thr Ser Val Gln Thr Ile
        115                 120                 125

Gln Leu Pro Gln Lys Pro Ile Ile Asp Thr Lys Leu Lys Asp Tyr Pro
    130                 135                 140

Lys Tyr Ser Pro Thr Gly Asn Ile Asp Asn Gly Thr Ser Pro Gln Leu
145                 150                 155                 160

Met Gly Trp Thr Leu Val Pro Cys Ile Met Val Asn Asp Pro Asn Ile
                165                 170                 175

Asp Lys Asn Thr Gln Ile Lys Thr Thr Pro Tyr Tyr Ile Leu Lys Lys
            180                 185                 190

Tyr Gln Tyr Trp Gln Arg Ala Val Gly Ser Asn Val Ala Leu Arg Pro
        195                 200                 205

His Glu Lys Lys Ser Tyr Thr Tyr Glu Trp Gly Thr Glu Ile Asp Gln
    210                 215                 220

Lys Thr Thr Ile Ile Asn Thr Leu Gly Phe Gln Ile Asn Ile Asp Ser
225                 230                 235                 240
```

Gly Met Lys Phe Asp Ile Pro Glu Val Gly Gly Gly Thr Asp Glu Ile
245 250 255

Lys Thr Gln Leu Asn Glu Glu Leu Lys Ile Glu Tyr Ser His Glu Thr
260 265 270

Lys Ile Met Glu Lys Tyr
275

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 829 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ACATGCAGCA ACTTATTTAA GTTTAGATGA TTCAGGTGTT AGTTTAATGA ATAAAAATGA 60

TGATGATATT GATGACTATA ATTTAAGGTG GTTTTTATTT CCTATTGATG ATAATCAATA 120

TATTATTACA AGCTACGCAG CGAATAATTG TAAGGTTTGG AATGTTAATA ATGATAAAAT 180

AAATGTTTCA ACTTATTCTT CAACAAACTC GATACAGAAA TGGCAAATAA AAGCTAATGC 240

TTCTTCGTAT GTAATACAAA GTAATAATGG GAAAGTTCTA ACAGCAGGAA CCGGTCAATC 300

TCTTGGATTA ATACGTTTAA CGGATGAATC ACCAGATAAT CCCAATCAAC AATGGAATTT 360

AACTCCTGTA CAAACAATTC AACTCCCACC AAAACCTACA ATAGATACAA AGTTAAAAGA 420

TTACCCCAAA TATTCACAAA CTGGCAATAT AGACAAGGGA ACACCTCCTC AATTAATGGG 480

ATGGACATTA ATACCTTGTA TTATGGTAAA TGATCCCAAT ATAGATAAAA ACACTCAAAT 540

CAAAACTACT CCATATTATA TTTTAAAAAA ATATCAATAT TGGCAACAAG CAGTAGGAAG 600

TAATGTAGCT TTACGTCCGC ATGAAAAAAA ATCATATGCT TATGAGTGGG GTACAGAAAT 660

AGATCAAAAA ACAACTATCA TTAATACATT AGGATTTCAG ATTAATATAG ATTCGGGAAT 720

GAAATTTGAT ATACCAGAAG TAGGTGGAGG TACAGATGAA ATAAAAACAC AATTAAACGA 780

AGAATTAAAA ATAGAATATA GCCGTGAAAC CAAAATAATG GAAAAATAT 829

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 276 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
His Ala Ala Thr Tyr Leu Ser Leu Asp Asp Ser Gly Val Ser Leu Met
1               5                  10                  15

Asn Lys Asn Asp Asp Asp Ile Asp Asp Tyr Asn Leu Arg Trp Phe Leu
            20                  25                  30

Phe Pro Ile Asp Asp Asn Gln Tyr Ile Ile Thr Ser Tyr Ala Ala Asn
        35                  40                  45

Asn Cys Lys Val Trp Asn Val Asn Asn Asp Lys Ile Asn Val Ser Thr
    50                  55                  60

Tyr Ser Ser Thr Asn Ser Ile Gln Lys Trp Gln Ile Lys Ala Asn Ala
65                  70                  75                  80

Ser Ser Tyr Val Ile Gln Ser Asn Asn Gly Lys Val Leu Thr Ala Gly
                85                  90                  95

Thr Gly Gln Ser Leu Gly Leu Ile Arg Leu Thr Asp Glu Ser Pro Asp
            100                 105                 110

Asn Pro Asn Gln Gln Trp Asn Leu Thr Pro Val Gln Thr Ile Gln Leu
        115                 120                 125

Pro Pro Lys Pro Thr Ile Asp Thr Lys Leu Lys Asp Tyr Pro Lys Tyr
    130                 135                 140

Ser Gln Thr Gly Asn Ile Asp Lys Gly Thr Pro Pro Gln Leu Met Gly
145                 150                 155                 160

Trp Thr Leu Ile Pro Cys Ile Met Val Asn Asp Pro Asn Ile Asp Lys
                165                 170                 175

Asn Thr Gln Ile Lys Thr Thr Pro Tyr Tyr Ile Leu Lys Lys Tyr Gln
            180                 185                 190

Tyr Trp Gln Gln Ala Val Gly Ser Asn Val Ala Leu Arg Pro His Glu
        195                 200                 205

Lys Lys Ser Tyr Ala Tyr Glu Trp Gly Thr Glu Ile Asp Gln Lys Thr
    210                 215                 220

Thr Ile Ile Asn Thr Leu Gly Phe Gln Ile Asn Ile Asp Ser Gly Met
225                 230                 235                 240

Lys Phe Asp Ile Pro Glu Val Gly Gly Gly Thr Asp Glu Ile Lys Thr
                245                 250                 255
```

Gln Leu Asn Glu Glu Leu Lys Ile Glu Tyr Ser Arg Glu Thr Lys Ile
260 265 270

Met Glu Lys Tyr
275

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Asp Ile Asp Asp Tyr Asn Leu
1 5

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Trp Phe Leu Phe Pro Ile Asp
1 5

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Gln Ile Lys Thr Thr Pro Tyr Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Tyr Glu Trp Gly Thr Glu
1 5

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GATATNGATG ANTAYAAYTT N 21

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TGGTTTTTNT TTCCNATNGA N 21

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CAAATNAAAA CNACNCCATA TTAT 24

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TANGANTGGG GNACAGAA 18

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

ATAATATGGN GTNGTTTTNA TTTG 24

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TTCTGTNCCC CANTCNTA 18

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CTCAAAGCGG ATCAGGAG 18

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GCGTATTCGG ATATGCTTGG 20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 386 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

Xaa Xaa Xaa Xaa Thr Tyr Leu Ser Leu Asp Asp Ser Gly Val Ser Leu
20 25 30

Met Xaa Lys Xaa Asp Xaa Asp Ile Asp Asp Tyr Asn Leu Xaa Trp Phe
35 40 45

Leu Phe Pro Ile Asp Xaa Xaa Gln Tyr Ile Ile Thr Ser Tyr Xaa Ala
50 55 60

Asn Asn Cys Lys Val Trp Asn Val Xaa Asn Asp Lys Ile Asn Val Ser
65 70 75 80

Thr Tyr Ser Ser Thr Asn Ser Xaa Gln Lys Trp Gln Ile Lys Ala Xaa
85 90 95

Xaa Ser Ser Tyr Xaa Ile Gln Ser Xaa Asn Gly Lys Val Leu Thr Ala
100 105 110

Gly Xaa Gly Gln Xaa Leu Gly Xaa Xaa Arg Leu Thr Asp Glu Xaa Xaa
115 120 125

Xaa Asn Xaa Asn Gln Gln Trp Asn Leu Thr Xaa Val Gln Thr Ile Gln
130 135 140

Leu Pro Xaa Lys Pro Xaa Ile Asp Xaa Lys Leu Lys Asp Xaa Pro Xaa
145 150 155 160

Tyr Ser Xaa Thr Gly Asn Ile Xaa Xaa Xaa Thr Xaa Pro Gln Leu Met
165 170 175

Gly Trp Thr Leu Xaa Pro Cys Ile Met Val Asn Asp Xaa Xaa Ile Asp
180 185 190

Lys Asn Thr Gln Ile Lys Thr Thr Pro Tyr Tyr Ile Xaa Lys Lys Tyr
195 200 205

Xaa Tyr Trp Xaa Xaa Ala Xaa Gly Ser Asn Val Xaa Leu Xaa Pro His
210 215 220

Xaa Lys Xaa Ser Tyr Xaa Tyr Glu Trp Gly Thr Glu Xaa Xaa Gln Lys
225 230 235 240

Thr Thr Ile Ile Asn Thr Xaa Gly Xaa Gln Ile Asn Ile Asp Ser Gly
245 250 255

Met Lys Phe Xaa Xaa Pro Glu Val Gly Gly Gly Thr Xaa Xaa Ile Lys
260 265 270

Thr Gln Leu Xaa Glu Glu Leu Lys Xaa Glu Tyr Ser Xaa Glu Thr Lys
275 280 285

Ile Met Xaa Lys Tyr Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
290 295 300

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
305 310 315 320

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
325 330 335

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
340 345 350

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
355 360 365

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
370 375 380

Xaa Xaa
385

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GNGAAGTNCA TATNGAAATN AATAATAC 28

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2015 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

ATTAATTTTA TGGAGGTTGA TATTTATGTC AGCTCGCGAA GTACACATTG AAATAAACAA 60

TAAAACACGT CATACATTAC AATTAGAGGA TAAAACTAAA CTTAGCGGCG GTAGATGGCG 120

AACATCACCT ACAAATGTTG CTCGTGATAC AATTAAAACA TTTGTAGCAG AATCACATGG 180

TTTTATGACA GGAGTAGAAG GTATTATATA TTTTAGTGTA AACGGAGACG CAGAAATTAG 240

TTTACATTTT GACAATCCTT ATATAGGTTC TAATAAATGT GATGGTTCTT CTGATAAACC 300

TGAATATGAA GTTATTACTC AAAGCGGATC AGGAGATAAA TCTCATGTGA CATATACTAT 360

TCAGACAGTA TCTTTACGAT TATAAGGAAA ATTTATAAAA ACTGTATTTT TTACTAAAAT 420

ACCAAAAAAT ACATATTTAT TTTTTGGTAT TTTCTAATAT GAAATATGAA TTATAAAAAT 480

ATTAATAAAA AAGGTGATAA AAATTATGTT AGATACTAAT AAAGTTTATG AAATAAGCAA 540

TCTTGCTAAT GGATTATATA CATCAACTTA TTTAAGTCTT GATGATTCAG GTGTTAGTTT 600

AATGAGTAAA AAGGATGAAG ATATTGATGA TTACAATTTA AAATGGTTTT TATTTCCTAT 660

TGATAATAAT CAATATATTA TTACAAGCTA TGGAGCTAAT AATTGTAAAG TTTGGAATGT 720

TAAAAATGAT AAAATAAATG TTTCAACTTA TTCTTCAACA AACTCTGTAC AAAAATGGCA 780

AATAAAAGCT AAAGATTCTT CATATATAAT ACAAAGTGAT AATGGAAAGG TCTTAACAGC 840

AGGAGTAGGT CAATCTCTTG GAATAGTACG CCTAACTGAT GAATTTCCAG AGAATTCTAA 900

CCAACAATGG AATTTAACTC CTGTACAAAC AATTCAACTC CCACAAAAAC CTAAAATAGA 960

TGAAAAATTA AAAGATCATC CTGAATATTC AGAAACCGGA AATATAAATC CTAAAACAAC 1020

TCCTCAATTA ATGGGATGGA CATTAGTACC TTGTATTATG GTAAATGATT CAAAAATAGA 1080

TAAAAACACT CAAATTAAAA CTACTCCATA TTATATTTTT AAAAAATATA AATACTGGAA 1140

TCTAGCAAAA GGAAGTAATG TATCTTTACT TCCACATCAA AAAAGATCAT ATGATTATGA 1200

ATGGGGTACA GAAAAAAATC AAAAAACAAC TATTATTAAT ACAGTAGGAT TGCAAATTAA 1260

TATAGATTCA GGAATGAAAT TTGAAGTACC AGAAGTAGGA GGAGGTACAG AAGACATAAA 1320

AACACAATTA ACTGAAGAAT TAAAAGTTGA ATATAGCACT GAAACCAAAA TAATGACGAA 1380

ATATCAAGAA CACTCAGAGA TAGATAATCC AACTAATCAA CCAATGAATT CTATAGGACT 1440

TCTTATTTAT ACTTCTTTAG AATTATATCG ATATAACGGT ACAGAAATTA AGATAATGGA 1500

CATAGAAACT TCAGATCATG ATACTTACAC TCTTACTTCT TATCCAAATC ATAAAGAAGC 1560

ATTATTACTT CTCACAAACC ATTCGTATGA AGAAGTAGAA GAAATAACAA AAATACCTAA 1620

GCATACACTT ATAAAATTGA AAAAACATTA TTTTAAAAAA TAAAAAACAT AATATATAAA 1680

TGACTGATTA ATATCTCTCG AAAAGGTTCT GGTGCAAAAA TAGTGGGATA TGAAAAAAGC 1740

AAAAGATTCC TAACGGAATG GAACATTAGG CTGTTAAATC AAAAAGTTTA TTGATAAAAT 1800

ATATCTGCCT TTGGACAGAC TTCTCCCCTT GGAGAGTTTG TCCTTTTTTG ACCATATGCA 1860

TAGCTTCTAT TCCGGCAATC ATTTTTGTAG CTGTTTGCAA GGATTTTAAT CCAAGCATAT 1920

CCGAATACGC TTTTTGATAA CCGATGTCTT GTTCAATGAT ATTGTTTAAT ATTTTCACAC 1980

GAATTGGCTA CTGTGCGGTA TCCTGTCTCC TTTAT 2015

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 360 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

ATGTCAGCTC GCGAAGTACA CATTGAAATA AACAATAAAA CACGTCATAC ATTACAATTA 60

GAGGATAAAA CTAAACTTAG CGGCGGTAGA TGGCGAACAT CACCTACAAA TGTTGCTCGT 120

GATACAATTA AAACATTTGT AGCAGAATCA CATGGTTTTA TGACAGGAGT AGAAGGTATT 180

ATATATTTTA GTGTAAACGG AGACGCAGAA ATTAGTTTAC ATTTTGACAA TCCTTATATA 240

GGTTCTAATA AATGTGATGG TTCTTCTGAT AAACCTGAAT ATGAAGTTAT TACTCAAAGC 300

GGATCAGGAG ATAAATCTCA TGTGACATAT ACTATTCAGA CAGTATCTTT ACGATTATAA 360

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```
Met Ser Ala Arg Glu Val His Ile Glu Ile Asn Asn Lys Thr Arg His
1               5                  10                  15

Thr Leu Gln Leu Glu Asp Lys Thr Lys Leu Ser Gly Gly Arg Trp Arg
            20                  25                  30

Thr Ser Pro Thr Asn Val Ala Arg Asp Thr Ile Lys Thr Phe Val Ala
        35                  40                  45

Glu Ser His Gly Phe Met Thr Gly Val Glu Gly Ile Ile Tyr Phe Ser
    50                  55                  60

Val Asn Gly Asp Ala Glu Ile Ser Leu His Phe Asp Asn Pro Tyr Ile
65                  70                  75                  80

Gly Ser Asn Lys Cys Asp Gly Ser Ser Asp Lys Pro Glu Tyr Glu Val
                85                  90                  95

Ile Thr Gln Ser Gly Ser Gly Asp Lys Ser His Val Thr Tyr Thr Ile
            100                 105                 110

Gln Thr Val Ser Leu Arg Leu
        115
```

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

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CATGAGATTT ATCTCCTGAT CCGC                                          24
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(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2230 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

ACTATGACAA TGATTATGAC TGCTGATGAA TTAGCTTTAT CAATACCAGG ATATTCTAAA 60

CCATCAAATA TAACAGGAGA TAAAAGTAAA CATACATTAT TTACTAATAT AATTGGAGAT 120

ATTCAAATAA AAGATCAAGC AACATTTGGG GTTGTTTTTG ATCCCCCTCT TAATCGTATT 180

TCAGGGGCTG AAGAATCAAG TAAGTTTATT GATGTATATT ATCCTTCTGA AGATAGTAAC 240

CTTAAATATT ATCAATTTAT AAAAGTAGCA ATTGATTTTG ATATTAATGA AGATTTTATT 300

AATTTTAATA ATCATGACAA TATAGGGATA TTTAATTTTG TTACACGAAA TTTTTTATTA 360

AATAATGAAA ATGATTAATA AAAAATTTAA TTTGTATAAT ATGTTTATTT TTTGAAAATT 420

GAATGCATAT ATTAATCGAG TATGTGTAAT AAATTTTAAT TTTATGGAGG TTGATATTTA 480

TGTCAGCACG TGAAGTACAC ATTGATGTAA ATAATAAGAC AGGTCATACA TTACAATTAG 540

AAGATAAAAC AAAACTTGAT GGTGGTAGAT GGCGAACATC ACCTACAAAT GTTGCTAATG 600

ATCAAATTAA AACATTTGTA GCAGAATCAC ATGGTTTTAT GACAGGTACA GAAGGTACTA 660

TATATTATAG TATAAATGGA GAAGCAGAAA TTAGTTTATA TTTTGACAAT CCTTATTCAG 720

GTTCTAATAA ATATGATGGG CATTCCAATA AAAATCAATA TGAAGTTATT ACCCAAGGAG 780

GATCAGGAAA TCAATCTCAT GTTACGTATA CTATTCAAAC TGTATCTTCA CGATATGGGA 840

ATAATTCATA AAAAAATATT TTTTTTTACG AAAATACCAA AAAAATTTTT TTGGTATTTT 900

CTAATATAAT TCATAAATAT TTTAATAATA AAATTATAAG AAAAGGTGAT AAATATTATG 960

TTAGATACTA ATAAAATTTA TGAAATAAGT AATTATGCTA ATGGATTACA TGCAGCAACT 1020

TATTTAAGTT TAGATGATTC AGGTGTTAGT TTAATGAATA AAAATGATGA TGATATTGAT 1080

GACTATAATT TAAGGTGGTT TTTATTTCCT ATTGATGATA ATCAATATAT TATTACAAGC 1140

TACGCAGCGA ATAATTGTAA GGTTTGGAAT GTTAATAATG ATAAAATAAA TGTTTCAACT 1200

TATTCTTCAA CAAACTCGAT ACAGAAATGG CAAATAAAAG CTAATGCTTC TTCGTATGTA 1260

ATACAAAGTA ATAATGGGAA AGTTCTAACA GCAGGAACCG GTCAATCTCT TGGATTAATA 1320

CGTTTAACGG ATGAATCACC AGATAATCCC AATCAACAAT GGAATTTAAC TCCTGTACAA 1380

ACAATTCAAC TCCCACCAAA ACCTACAATA GATACAAAGT TAAAAGATTA CCCCAAATAT 1440

TCACAAACTG GCAATATAGA CAAGGGAACA CCTCCTCAAT TAATGGGATG GACATTAATA 1500

CCTTGTATTA TGGTAAATGA TCCAAATATA GATAAAAACA CTCAAATCAA AACTACTCCA 1560

TATTATATTT TAAAAAAATA TCAATATTGG CAACAAGCAG TAGGAAGTAA TGTAGCTTTA 1620

CGTCCGCATG AAAAAAAATC ATATGCTTAT GAGTGGGGTA CAGAAATAGA TCAAAAAACA 1680

ACTATCATTA ATACATTAGG ATTTCAGATT AATATAGATT CGGGAATGAA ATTTGATATA 1740

CCAGAAGTAG GTGGAGGTAC AGATGAAATA AAAACACAAT TAAACGAAGA ATTAAAAATA 1800

GAATATAGCC GTGAAACCAA AATAATGGAA AAATATCAGG AACAATCAGA GATAGATAAT 1860

CCAACTGATC AATCAATGAA TTCTATAGGA TTCCTCACTA TTACTTCTTT AGAATTATAT 1920

CGATATAATG GTTCGGAAAT TAGTGTAATG AAAATTCAAA CTTCAGATAA TGATACTTAC 1980

AATGTGACCT CTTATCCAGA TCATCAACAA GCTCTATTAC TTCTTACAAA TCATTCATAT 2040

GAAGAAGTAG AAGAAATAAC AAATATTCCC AAAATATCAC TGAAAAAATT AAAAAAATAT 2100

TATTTTTAAA ACATAATTAT ATTTTGATAG CTTTTTAAAA ATAAAGATTG TTCAAAGTAA 2160

AATGAAAGAA AATCTTTTAT GAAACTTTAA TACAATAAAA GAGGAATATT TTCTTATAAG 2220

TACTTCCTTG 2230

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 372 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

ATGTCAGCAC GTGAAGTACA CATTGATGTA AATAATAAGA CAGGTCATAC ATTACAATTA 60

GAAGATAAAA CAAAACTTGA TGGTGGTAGA TGGCGAACAT CACCTACAAA TGTTGCTAAT 120

GATCAAATTA AAACATTTGT AGCAGAATCA CATGGTTTTA TGACAGGTAC AGAAGGTACT 180

ATATATTATA GTATAAATGG AGAAGCAGAA ATTAGTTTAT ATTTTGACAA TCCTTATTCA 240

GGTTCTAATA AATATGATGG GCATTCCAAT AAAAATCAAT ATGAAGTTAT TACCCAAGGA 300

GGATCAGGAA ATCAATCTCA TGTTACGTAT ACTATTCAAA CTGTATCTTC ACGATATGGG 360

AATAATTCAT AA 372

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 123 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Met Ser Ala Arg Glu Val His Ile Asp Val Asn Asn Lys Thr Gly His
1 5 10 15

Thr Leu Gln Leu Glu Asp Lys Thr Lys Leu Asp Gly Gly Arg Trp Arg
20 25 30

Thr Ser Pro Thr Asn Val Ala Asn Asp Gln Ile Lys Thr Phe Val Ala
35 40 45

Glu Ser His Gly Phe Met Thr Gly Thr Glu Gly Thr Ile Tyr Tyr Ser
50 55 60

Ile Asn Gly Glu Ala Glu Ile Ser Leu Tyr Phe Asp Asn Pro Tyr Ser
65 70 75 80

Gly Ser Asn Lys Tyr Asp Gly His Ser Asn Lys Asn Gln Tyr Glu Val
85 90 95

Ile Thr Gln Gly Gly Ser Gly Asn Gln Ser His Val Thr Tyr Thr Ile
100 105 110

Gln Thr Val Ser Ser Arg Tyr Gly Asn Asn Ser
115 120

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1152 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

ATGTTAGATA CTAATAAAAT TTATGAAATA AGTAATTATG CTAATGGATT ACATGCAGCA 60

ACTTATTTAA GTTTAGATGA TTCAGGTGTT AGTTTAATGA ATAAAAATGA TGATGATATT 120

GATGACTATA ATTTAAGGTG GTTTTTATTT CCTATTGATG ATAATCAATA TATTATTACA 180

AGCTACGCAG CGAATAATTG TAAGGTTTGG AATGTTAATA ATGATAAAAT AAATGTTTCA 240

ACTTATTCTT CAACAAACTC GATACAGAAA TGGCAAATAA AAGCTAATGC TTCTTCGTAT 300

GTAATACAAA GTAATAATGG GAAAGTTCTA ACAGCAGGAA CCGGTCAATC TCTTGGATTA 360

ATACGTTTAA CGGATGAATC ACCAGATAAT CCCAATCAAC AATGGAATTT AACTCCTGTA 420

CAAACAATTC AACTCCCACC AAAACCTACA ATAGATACAA AGTTAAAAGA TTACCCCAAA 480

TATTCACAAA CTGGCAATAT AGACAAGGGA ACACCTCCTC AATTAATGGG ATGGACATTA 540

ATACCTTGTA TTATGGTAAA TGATCCAAAT ATAGATAAAA ACACTCAAAT CAAAACTACT 600

CCATATTATA TTTTAAAAAA ATATCAATAT TGGCAACAAG CAGTAGGAAG TAATGTAGCT 660

TTACGTCCGC ATGAAAAAAA ATCATATGCT TATGAGTGGG GTACAGAAAT AGATCAAAAA 720

ACAACTATCA TTAATACATT AGGATTTCAG ATTAATATAG ATTCGGGAAT GAAATTTGAT 780

ATACCAGAAG TAGGTGGAGG TACAGATGAA ATAAAAACAC AATTAAACGA AGAATTAAAA 840

ATAGAATATA GCCGTGAAAC CAAAATAATG GAAAAATATC AGGAACAATC AGAGATAGAT 900

AATCCAACTG ATCAATCAAT GAATTCTATA GGATTCCTCA CTATTACTTC TTTAGAATTA 960

TATCGATATA ATGGTTCGGA AATTAGTGTA ATGAAAATTC AAACTTCAGA TAATGATACT 1020

TACAATGTGA CCTCTTATCC AGATCATCAA CAAGCTCTAT TACTTCTTAC AAATCATTCA 1080

TATGAAGAAG TAGAAGAAAT AACAAATATT CCCAAAATAT CACTGAAAAA ATTAAAAAAA 1140

TATTATTTTT AA 1152

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 383 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Met Leu Asp Thr Asn Lys Ile Tyr Glu Ile Ser Asn Tyr Ala Asn Gly
1 5 10 15

Leu His Ala Ala Thr Tyr Leu Ser Leu Asp Asp Ser Gly Val Ser Leu
20 25 30

Met Asn Lys Asn Asp Asp Asp Ile Asp Asp Tyr Asn Leu Arg Trp Phe
35 40 45

Leu Phe Pro Ile Asp Asp Asn Gln Tyr Ile Ile Thr Ser Tyr Ala Ala
50 55 60

Asn Asn Cys Lys Val Trp Asn Val Asn Asn Asp Lys Ile Asn Val Ser
65 70 75 80

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Thr Tyr Ser Ser Thr Asn Ser Ile Gln Lys Trp Gln Ile Lys Ala Asn
                85                  90                  95

Ala Ser Ser Tyr Val Ile Gln Ser Asn Asn Gly Lys Val Leu Thr Ala
            100                 105                 110

Gly Thr Gly Gln Ser Leu Gly Leu Ile Arg Leu Thr Asp Glu Ser Pro
        115                 120                 125

Asp Asn Pro Asn Gln Gln Trp Asn Leu Thr Pro Val Gln Thr Ile Gln
    130                 135                 140

Leu Pro Pro Lys Pro Thr Ile Asp Thr Lys Leu Lys Asp Tyr Pro Lys
145                 150                 155                 160

Tyr Ser Gln Thr Gly Asn Ile Asp Lys Gly Thr Pro Pro Gln Leu Met
                165                 170                 175

Gly Trp Thr Leu Ile Pro Cys Ile Met Val Asn Asp Pro Asn Ile Asp
            180                 185                 190

Lys Asn Thr Gln Ile Lys Thr Thr Pro Tyr Tyr Ile Leu Lys Lys Tyr
        195                 200                 205

Gln Tyr Trp Gln Gln Ala Val Gly Ser Asn Val Ala Leu Arg Pro His
    210                 215                 220

Glu Lys Lys Ser Tyr Ala Tyr Glu Trp Gly Thr Glu Ile Asp Gln Lys
225                 230                 235                 240

Thr Thr Ile Ile Asn Thr Leu Gly Phe Gln Ile Asn Ile Asp Ser Gly
                245                 250                 255

Met Lys Phe Asp Ile Pro Glu Val Gly Gly Gly Thr Asp Glu Ile Lys
            260                 265                 270

Thr Gln Leu Asn Glu Glu Leu Lys Ile Glu Tyr Ser Arg Glu Thr Lys
        275                 280                 285

Ile Met Glu Lys Tyr Gln Glu Gln Ser Glu Ile Asp Asn Pro Thr Asp
    290                 295                 300

Gln Ser Met Asn Ser Ile Gly Phe Leu Thr Ile Thr Ser Leu Glu Leu
305                 310                 315                 320

Tyr Arg Tyr Asn Gly Ser Glu Ile Ser Val Met Lys Ile Gln Thr Ser
                325                 330                 335

Asp Asn Asp Thr Tyr Asn Val Thr Ser Tyr Pro Asp His Gln Gln Ala
            340                 345                 350

Leu Leu Leu Leu Thr Asn His Ser Tyr Glu Glu Val Glu Glu Ile Thr
        355                 360                 365

Asn Ile Pro Lys Ile Ser Leu Lys Lys Leu Lys Lys Tyr Tyr Phe
    370                 375                 380
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(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2132 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GTATTTCAGG GGGTGAAGAT TCAAGTAAGT TTATTGATGT ATATTATCCT TTTGAAGATA 60

GTAATTTTAA ATATTATCAA TTTATAAAAG TAGCAATTGA TTTTGATATT AATGAAGATT 120

TTATTAATTT TAATAATCAT GACAATATAG GGATATTTAA TTTTGTTACA CGAAATTTTT 180

TATTAAATAA TGAAAATGAT GAATAAAAAA TTTAATTTGT TTATTATGTT TATTTTTTGA 240

AAATTGAATG CATATATTAA TCGAGTATGT ATAATAAATT TTAATTTTAT GGAGGTTGAT 300

ATTTATGTCA GCACGTGAAG TACACATTGA TGTAAATAAT AAGACAGGTC ATACATTACA 360

ATTAGAAGAT AAAACAAAAC TTGATGGTGG TAGATGGCGA ACATCACCTA CAAATGTTGC 420

TAATGATCAA ATTAAAACAT TTGTAGCAGA ATCAAATGGT TTTATGACAG GTACAGAAGG 480

TACTATATAT TATAGTATAA ATGGAGAAGC AGAAATTAGT TTATATTTTG ACAATCCTTT 540

TGCAGGTTCT AATAAATATG ATGGACATTC CAATAAATCT CAATATGAAA TTATTACCCA 600

AGGAGGATCA GGAAATCAAT CTCATGTTAC GTATACTATT CAAACCACAT CCTCACGATA 660

TGGGCATAAA TCATAACAAA TAATTTTTTA CGAAAATACC AAAAAATAAA TATTTTTTGG 720

TATTTTCTAA TATAAATTAC AAATATATTA ATAATAAAAT TATAAGAAAA GGTGATAAAG 780

ATTATGTTAG ATACTAATAA AGTTTATGAA ATAAGCAATC ATGCTAATGG ACTATATGCA 840

GCAACTTATT TAAGTTTAGA TGATTCAGGT GTTAGTTTAA TGAATAAAAA TGATGATGAT 900

ATTGATGATT ATAACTTAAA ATGGTTTTTA TTTCCTATTG ATGATGATCA ATATATTATT 960

ACAAGCTATG CAGCAAATAA TTGTAAAGTT TGGAATGTTA ATAATGATAA AATAAATGTT 1020

TCGACTTATT CTTCAACAAA TTCAATACAA AAATGGCAAA TAAAAGCTAA TGGTTCTTCA 1080

TATGTAATAC AAAGTGATAA TGGAAAAGTC TTAACAGCAG GAACCGGTCA AGCTCTTGGA 1140

TTGATACGTT TAACTGATGA ATCCTCAAAT AATCCCAATC AACAATGGAA TTTAACTTCT 1200

GTACAAACAA TTCAACTTCC ACAAAAACCT ATAATAGATA CAAAATTAAA AGATTATCCC 1260

AAATATTCAC CAACTGGAAA TATAGATAAT GGAACATCTC CTCAATTAAT GGGATGGACA 1320

TTAGTACCTT GTATTATGGT AAATGATCCA AATATAGATA AAAATACTCA AATTAAAACT 1380

ACTCCATATT ATATTTTAAA AAAATATCAA TATTGGCAAC GAGCAGTAGG AAGTAATGTA 1440

GCTTTACGTC CACATGAAAA AAAATCATAT ACTTATGAAT GGGGCACAGA AATAGATCAA 1500

AAAACAACAA TTATAAATAC ATTAGGATTT CAAATCAATA TAGATTCAGG AATGAAATTT 1560

GATATACCAG AAGTAGGTGG AGGTACAGAT GAAATAAAAA CACAACTAAA TGAAGAATTA 1620

AAAATAGAAT ATAGTCATGA AACTAAAATA ATGGAAAAAT ATCAAGAACA ATCTGAAATA 1680

GATAATCCAA CTGATCAATC AATGAATTCT ATAGGATTTC TTACTATTAC TTCCTTAGAA 1740

TTATATAGAT ATAATGGCTC AGAAATTCGT ATAATGCAAA TTCAAACCTC AGATAATGAT 1800

ACTTATAATG TTACTTCTTA TCCAAATCAT CAACAAGCTT TATTACTTCT TACAAATCAT 1860

TCATATGAAG AAGTAGAAGA AATAACAAAT ATTCCTAAAA GTACACTAAA AAAATTAAAA 1920

AAATATTATT TTTAAATATT GAAATTAGAA ATTATCTAAA ACAAAACGAA AGATAATTTA 1980

ATCTTTAATT ATTTGTAAGA TAATCGTATT TTATTTGTAT TAATTTTTAT ACAATATAAA 2040

GTAATATCTG TACGTGAAAT TGGTTTCGCT TCAATATCTA ATCTCATCTC ATGTATTACA 2100

TGCGTAATAC CTTCTTGTTC TGCTTCTACA AG 2132

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 372 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

ATGTCAGCAC GTGAAGTACA CATTGATGTA AATAATAAGA CAGGTCATAC ATTACAATTA 60

GAAGATAAAA CAAAACTTGA TGGTGGTAGA TGGCGAACAT CACCTACAAA TGTTGCTAAT 120

GATCAAATTA AAACATTTGT AGCAGAATCA AATGGTTTTA TGACAGGTAC AGAAGGTACT 180

ATATATTATA GTATAAATGG AGAAGCAGAA ATTAGTTTAT ATTTTGACAA TCCTTTTGCA 240

GGTTCTAATA AATATGATGG ACATTCCAAT AAATCTCAAT ATGAAATTAT TACCCAAGGA 300

GGATCAGGAA ATCAATCTCA TGTTACGTAT ACTATTCAAA CCACATCCTC ACGATATGGG 360

CATAAATCAT AA 372

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 123 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Met Ser Ala Arg Glu Val His Ile Asp Val Asn Asn Lys Thr Gly His
1 5 10 15

Thr Leu Gln Leu Glu Asp Lys Thr Lys Leu Asp Gly Gly Arg Trp Arg
20 25 30

Thr Ser Pro Thr Asn Val Ala Asn Asp Gln Ile Lys Thr Phe Val Ala
35 40 45

Glu Ser Asn Gly Phe Met Thr Gly Thr Glu Gly Thr Ile Tyr Tyr Ser
50 55 60

Ile Asn Gly Glu Ala Glu Ile Ser Leu Tyr Phe Asp Asn Pro Phe Ala
65 70 75 80

Gly Ser Asn Lys Tyr Asp Gly His Ser Asn Lys Ser Gln Tyr Glu Ile
85 90 95

Ile Thr Gln Gly Gly Ser Gly Asn Gln Ser His Val Thr Tyr Thr Ile
100 105 110

Gln Thr Thr Ser Ser Arg Tyr Gly His Lys Ser
115 120

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1152 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

ATGTTAGATA CTAATAAAGT TTATGAAATA AGCAATCATG CTAATGGACT ATATGCAGCA 60

ACTTATTTAA GTTTAGATGA TTCAGGTGTT AGTTTAATGA ATAAAAATGA TGATGATATT 120

GATGATTATA ACTTAAAATG GTTTTTATTT CCTATTGATG ATGATCAATA TATTATTACA 180

AGCTATGCAG CAAATAATTG TAAAGTTTGG AATGTTAATA ATGATAAAAT AAATGTTTCG 240

ACTTATTCTT CAACAAATTC AATACAAAAA TGGCAAATAA AAGCTAATGG TTCTTCATAT 300

GTAATACAAA GTGATAATGG AAAAGTCTTA ACAGCAGGAA CCGGTCAAGC TCTTGGATTG 360

ATACGTTTAA CTGATGAATC CTCAAATAAT CCCAATCAAC AATGGAATTT AACTTCTGTA 420

CAAACAATTC AACTTCCACA AAAACCTATA ATAGATACAA AATTAAAAGA TTATCCCAAA 480

TATTCACCAA CTGGAAATAT AGATAATGGA ACATCTCCTC AATTAATGGG ATGGACATTA 540

GTACCTTGTA TTATGGTAAA TGATCCAAAT ATAGATAAAA ATACTCAAAT TAAAACTACT 600

CCATATTATA TTTTAAAAAA ATATCAATAT TGGCAACGAG CAGTAGGAAG TAATGTAGCT 660

TTACGTCCAC ATGAAAAAAA ATCATATACT TATGAATGGG GCACAGAAAT AGATCAAAAA 720

ACAACAATTA TAAATACATT AGGATTTCAA ATCAATATAG ATTCAGGAAT GAAATTTGAT 780

ATACCAGAAG TAGGTGGAGG TACAGATGAA ATAAAAACAC AACTAAATGA AGAATTAAAA 840

ATAGAATATA GTCATGAAAC TAAAATAATG GAAAAATATC AAGAACAATC TGAAATAGAT 900

AATCCAACTG ATCAATCAAT GAATTCTATA GGATTTCTTA CTATTACTTC CTTAGAATTA 960

TATAGATATA ATGGCTCAGA AATTCGTATA ATGCAAATTC AAACCTCAGA TAATGATACT 1020

TATAATGTTA CTTCTTATCC AAATCATCAA CAAGCTTTAT TACTTCTTAC AAATCATTCA 1080

TATGAAGAAG TAGAAGAAAT AACAAATATT CCTAAAAGTA CACTAAAAAA ATTAAAAAAA 1140

TATTATTTTT AA 1152

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 383 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Met Leu Asp Thr Asn Lys Val Tyr Glu Ile Ser Asn His Ala Asn Gly
1 5 10 15

Leu Tyr Ala Ala Thr Tyr Leu Ser Leu Asp Asp Ser Gly Val Ser Leu
20 25 30

Met Asn Lys Asn Asp Asp Asp Ile Asp Asp Tyr Asn Leu Lys Trp Phe
35 40 45

Leu Phe Pro Ile Asp Asp Asp Gln Tyr Ile Ile Thr Ser Tyr Ala Ala
50 55 60

Asn Asn Cys Lys Val Trp Asn Val Asn Asn Asp Lys Ile Asn Val Ser
65 70 75 80

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Thr Tyr Ser Ser Thr Asn Ser Ile Gln Lys Trp Gln Ile Lys Ala Asn
                85                  90                  95

Gly Ser Ser Tyr Val Ile Gln Ser Asp Asn Gly Lys Val Leu Thr Ala
            100                 105                 110

Gly Thr Gly Gln Ala Leu Gly Leu Ile Arg Leu Thr Asp Glu Ser Ser
        115                 120                 125

Asn Asn Pro Asn Gln Gln Trp Asn Leu Thr Ser Val Gln Thr Ile Gln
    130                 135                 140

Leu Pro Gln Lys Pro Ile Ile Asp Thr Lys Leu Lys Asp Tyr Pro Lys
145                 150                 155                 160

Tyr Ser Pro Thr Gly Asn Ile Asp Asn Gly Thr Ser Pro Gln Leu Met
                165                 170                 175

Gly Trp Thr Leu Val Pro Cys Ile Met Val Asn Asp Pro Asn Ile Asp
            180                 185                 190

Lys Asn Thr Gln Ile Lys Thr Thr Pro Tyr Tyr Ile Leu Lys Lys Tyr
        195                 200                 205

Gln Tyr Trp Gln Arg Ala Val Gly Ser Asn Val Ala Leu Arg Pro His
    210                 215                 220

Glu Lys Lys Ser Tyr Thr Tyr Glu Trp Gly Thr Glu Ile Asp Gln Lys
225                 230                 235                 240

Thr Thr Ile Ile Asn Thr Leu Gly Phe Gln Ile Asn Ile Asp Ser Gly
                245                 250                 255

Met Lys Phe Asp Ile Pro Glu Val Gly Gly Gly Thr Asp Glu Ile Lys
            260                 265                 270

Thr Gln Leu Asn Glu Glu Leu Lys Ile Glu Tyr Ser His Glu Thr Lys
        275                 280                 285

Ile Met Glu Lys Tyr Gln Glu Gln Ser Glu Ile Asp Asn Pro Thr Asp
    290                 295                 300

Gln Ser Met Asn Ser Ile Gly Phe Leu Thr Ile Thr Ser Leu Glu Leu
305                 310                 315                 320

Tyr Arg Tyr Asn Gly Ser Glu Ile Arg Ile Met Gln Ile Gln Thr Ser
                325                 330                 335

Asp Asn Asp Thr Tyr Asn Val Thr Ser Tyr Pro Asn His Gln Gln Ala
            340                 345                 350

Leu Leu Leu Leu Thr Asn His Ser Tyr Glu Glu Val Glu Glu Ile Thr
        355                 360                 365

Asn Ile Pro Lys Ser Thr Leu Lys Lys Leu Lys Lys Tyr Tyr Phe
    370                 375                 380
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(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 360 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

ATGTCCGCCC GCGAGGTGCA CATCGAGATC AACAACAAGA CCCGCCACAC CCTCCAGCTC 60

GAGGACAAGA CCAAGCTCTC CGGCGGCAGG TGGCGCACCT CCCCGACCAA CGTGGCCCGC 120

GACACCATCA AGACGTTCGT GGCGGAGTCC CACGGCTTCA TGACCGGCGT CGAGGGCATC 180

ATCTACTTCT CCGTGAACGG CGACGCCGAG ATCTCCCTCC ACTTCGACAA CCCGTACATC 240

GGCTCCAACA AGTGCGACGG CTCCTCCGAC AAGCCCGAGT ACGAGGTGAT CACCCAGTCC 300

GGCTCCGGCG ACAAGTCCCA CGTGACCTAC ACCATCCAGA CCGTGTCCCT CCGCCTCTGA 360

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1158 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

ATGCTCGACA CCAACAAGGT GTACGAGATC TCCAACCTCG CCAACGGCCT CTACACCTCC 60

ACCTACCTCT CCCTCGACGA CTCCGGCGTG TCCCTCATGT CCAAGAAGGA CGAGGACATC 120

GACGACTACA ACCTCAAGTG GTTCCTCTTC CCGATCGACA ACAACCAGTA CATCATCACC 180

TCCTACGGCG CCAACAACTG CAAGGTGTGG AACGTGAAGA ACGACAAGAT CAACGTGTCC 240

ACCTACTCCT CCACCAACTC CGTGCAGAAG TGGCAGATCA AGGCCAAGGA CTCCTCCTAC 300

ATCATCCAGT CCGACAACGG CAAGGTGCTC ACCGCGGGCG TGGGCCAGTC CCTCGGCATC 360

GTGCGCCTCA CCGACGAGTT CCCGGAGAAC TCCAACCAGC AATGGAACCT CACCCCGGTG 420

CAGACCATCC AGCTCCCGCA GAAGCCGAAG ATCGACGAGA AGCTCAAGGA CCACCCGGAG 480

TACTCCGAGA CCGGCAACAT CAACCCGAAG ACCACCCCGC AGCTCATGGG CTGGACCCTC 540

GTGCCGTGCA TCATGGTGAA CGACTCCAAG ATCGACAAGA ACACCCAGAT CAAGACCACC 600

CCGTACTACA TCTTCAAGAA ATACAAGTAC TGGAACCTCG CCAAGGGCTC CAACGTGTCC 660

CTCCTCCCGC ACCAGAAGCG CAGCTACGAC TACGAGTGGG GCACCGAGAA GAACCAGAAG 720

ACCACCATCA TCAACACCGT GGGCCTGCAG ATCAACATCG ACTCGGGGAT GAAGTTCGAG 780

GTGCCGGAGG TGGGCGGCGG CACCGAGGAC ATCAAGACCC AGCTCACCGA GGAGCTGAAG 840

GTGGAGTACT CCACCGAGAC CAAGATCATG ACCAAGTACC AGGAGCACTC CGAGATCGAC 900

AACCCGACCA ACCAGCCGAT GAACTCCATC GGCCTCCTCA TCTACACCTC CCTCGAGCTG 960

TACCGCTACA ACGGCACCGA GATCAAGATC ATGGACATCG AGACCTCCGA CCACGACACC 1020

TACACCCTCA CCTCCTACCC GAACCACAAG GAGGCGCTGC TGCTGCTGAC CAACCACTCC 1080

TACGAGGAGG TGGAGGAGAT CACCAAGATC CCGAAGCACA CCCTCATCAA GCTCAAGAAG 1140

CACTACTTCA AGAAGTGA 1158

Claims

1. An isolated polynucleotide comprising a nucleotide sequence which encodes a toxin active against a non-mammalian pest wherein said nucleotide sequence hybridizes under stringent conditions with a nucleotide sequence selected from the group consisting of: DNA which encodes SEQ ID NO. 2; DNA which encodes SEQ ID NO. 4; DNA which encodes SEQ ID NO. 6; SEQ ID NO. 8; SEQ ID NO. 10; DNA which encodes SEQ ID NO. 11; SEQ ID NO. 12; DNA which encodes SEQ ID NO. 13; SEQ ID NO. 14; DNA which encodes SEQ ID NO. 15; DNA which encodes SEQ ID NO. 16; DNA which encodes SEQ ID NO. 17; DNA which encodes SEQ ID NO. 18; DNA which encodes SEQ ID NO. 19; SEQ ID NO. 20; SEQ ID NO. 21; SEQ ID NO. 22; SEQ ID NO. 23; SEQ ID NO. 24; SEQ ID NO. 25; SEQ ID NO. 26; SEQ ID NO. 27; DNA which encodes a pesticidal portion of SEQ ID NO. 28; SEQ ID NO. 37; DNA which encodes SEQ ID NO. 38; SEQ ID NO. 42; and DNA which encodes SEQ ID NO. 43.

2. The isolated polynucleotide, according to claim 1, wherein said nucleotide sequence hybridizes with DNA which encodes SEQ ID NO. 2.

3. The isolated polynucleotide, according to claim 1, wherein said nucleotide sequence hybridizes with SEQ ID NO. 10.

4. The isolated polynucleotide, according to claim 1, wherein said toxin has a molecular weight of approximately 40-50 kDa.

5. An isolated polynucleotide comprising a nucleotide sequence which encodes a toxin active against a non-mammalian pest wherein said toxin immunoreacts with an antibody to an approximately 40-50 kDa toxin from a *Bacillus thuringiensis* isolate selected from the group consisting of PS80JJ1, having the identifying characteristics of NRRL B-18679; PS149B1, having the identifying characteristics of NRRL B-21553; and PS167H2, having the identifying characteristics of NRRL B-21554.

6. The isolated polynucleotide, according to claim 5, wherein said nucleotide sequence encodes a toxin of approximately 40-50 kDa.

7. An isolated polynucleotide comprising a nucleotide sequence which encodes a toxin active against a non-mammalian pest wherein a portion of said nucleotide sequence can be amplified by PCR using a primer pair selected from the following group:

SEQ ID NOS. 20 and 24 to produce a fragment of about 495 bp; SEQ ID NOS. 20 and 25 to produce a fragment of about 594 bp; SEQ ID NOS. 21 and 24 to produce a fragment of about 471 bp; and SEQ ID NOS. 21 and 25 to produce a fragment of about 580 bp.

8. The isolated polynucleotide, according to claim 7, wherein said nucleotide sequence encodes a toxin of approximately 40-50 kDa.

9. An isolated polynucleotide comprising a nucleotide sequence which encodes a toxin active against a non-mammalian pest wherein said toxin comprises a pesticidal portion of an amino acid sequence shown in the group selected from SEQ ID NO. 30, SEQ ID NO. 34, and SEQ ID NO. 39.

10. The isolated polynucleotide, according to claim 9, wherein said nucleotide sequence encodes a toxin which comprises a pesticidal portion of the consensus sequence shown in Figure 1.

11. The isolated polynucleotide, according to claim 9, wherein said nucleotide sequence encodes a toxin of approximately 40-50 kDa.

12. An isolated polynucleotide comprising a nucleotide sequence which encodes a toxin active against a non-mammalian pest wherein said toxin comprises an amino acid sequence which has at least about 75% homology with a pesticidal portion of an amino acid sequence selected from the group consisting of SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 38, and SEQ ID NO. 43.

13. The isolated polynucleotide, according to claim 12, wherein said nucleotide sequence encodes a toxin which comprises an amino acid sequence which has at least about 80% homology with a pesticidal portion of an amino acid sequence selected from the group consisting of SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 38, and SEQ ID NO. 43.

14. The isolated polynucleotide, according to claim 12, wherein said nucleotide sequence encodes a toxin which comprises an amino acid sequence which has at least about 90% homology with a pesticidal portion of an amino acid sequence selected from the group consisting of SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 38, and SEQ ID NO. 43.

15. An isolated polynucleotide comprising a nucleotide sequence which encodes a toxin which is active against a non-mammalian pest, wherein said nucleotide sequence is from a *Bacillus thuringiensis* isolate selected from the group consisting of PS80JJ1, having the identifying characteristics of NRRL B-18679; PS149B1, having the identifying characteristics of NRRL B-21553; and PS167H2, having the identifying characteristics of NRRL B-21554; and mutants thereof which retain pesticidal activity.

16. The isolated polynucleotide, according to claim 15, wherein said toxin is approximately 40-50 kDa.

17. The isolated polynucleotide, according to claim 15, wherein said toxin is approximately 10-15 kDa.

18. An isolated polynucleotide comprising a nucleotide sequence which encodes a toxin active against a non-mammalian pest wherein said nucleotide sequence hybridizes under stringent conditions with a nucleotide sequence selected from the group consisting of: DNA which encodes SEQ ID NO. 3; DNA which encodes SEQ ID NO. 5; and DNA which encodes SEQ ID NO. 7.

19. The isolated polynucleotide, according to claim 18, wherein said nucleotide sequence encodes a toxin of about 10-15 kDa.

20. An isolated polynucleotide comprising a nucleotide sequence which encodes a toxin active against a non-mammalian pest wherein said toxin immunoreacts with an antibody to an approximately 10-15 kDa toxin, or a fragment thereof, from a *Bacillus thuringiensis* isolate selected from the group consisting of PS80JJ1, having the identifying characteristics of NRRL B-18679; PS149B1, having the identifying characteristics of NRRL B-21553; and PS167H2, having the identifying characteristics of NRRL B-21554.

21. The isolated polynucleotide, according to claim 20, wherein said nucleotide sequence encodes a toxin of approximately 10-15 kDa.

22. An isolated polynucleotide comprising a nucleotide sequence which encodes a toxin active against a non-mammalian pest wherein a portion of said nucleotide sequence can be amplified by PCR using the primer pair of SEQ ID NO. 29 and SEQ ID NO. 33.

23. The isolated polynucleotide, according to claim 22, wherein said nucleotide sequence encodes a toxin of approximately 10-15 kDa.

24. An isolated polynucleotide comprising a nucleotide sequence which encodes a toxin active against a non-mammalian pest wherein said toxin comprises a pesticidal portion of an amino acid sequence selected from the group consisting of SEQ ID NO. 32, SEQ ID NO. 36, and SEQ ID NO. 41.

25. The isolated polynucleotide, according to claim 24, wherein said toxin comprises the amino acid sequence shown in SEQ ID NO. 32.

26. The isolated polynucleotide, according to claim 24, wherein said nucleotide sequence encodes a toxin of approximately 10-15 kDa.

27. An isolated polynucleotide comprising a nucleotide sequence which encodes a toxin active against a non-mammalian pest wherein said toxin comprises an amino acid sequence which has at least about 75% homology with an amino acid sequence selected from the group consisting of SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of SEQ ID NO. 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of sequence IDS NO. 41.

28. The isolated polynucleotide, according to claim 27, wherein said nucleotide sequence encodes a toxin which comprises an amino acid sequence which has at least about 80% homology with an amino acid sequence selected from the group consisting of SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of SEQ ID NO. 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of sequence IDS NO. 41.

29. The isolated polynucleotide, according to claim 27, wherein said nucleotide sequence encodes a toxin which comprises an amino acid sequence which has at least about 90% homology with an amino acid sequence selected from the group consisting of SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of SEQ ID NO. 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of sequence IDS NO. 41.

30. A purified toxin active against a non-mammalian pest, wherein said toxin is encoded by a nucleotide sequence which hybridizes under stringent conditions with a nucleotide sequence selected from the group consisting of: DNA which encodes SEQ ID NO. 2; DNA which encodes SEQ ID NO. 4; DNA which encodes SEQ ID NO. 6; SEQ ID NO. 8; SEQ ID NO. 10; DNA which encodes SEQ ID NO. 11; SEQ ID NO. 12; DNA which encodes SEQ ID NO. 13; SEQ ID NO. 14; DNA which encodes SEQ ID NO. 15; DNA which encodes SEQ ID NO. 16; DNA which encodes SEQ ID NO. 17; DNA which encodes SEQ ID NO. 18; DNA which encodes SEQ ID NO. 19; SEQ ID NO. 20; SEQ ID NO. 21; SEQ ID NO. 22; SEQ ID NO. 23; SEQ ID NO. 24; SEQ ID NO. 25; SEQ ID NO. 26; SEQ ID NO. 27; DNA which encodes a pesticidal portion of SEQ ID NO. 28, SEQ ID NO. 37, DNA which encodes SEQ ID NO. 38, SEQ ID NO. 42, and DNA which encodes SEQ ID NO. 43.

31. The purified toxin, according to claim 30, wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11.

32. The purified toxin, according to claim 31, wherein said toxin is encoded by a nucleotide sequence which hybridizes with DNA which encodes SEQ ID NO. 2.

33. The purified toxin, according to claim 31, which is encoded by DNA which hybridizes with SEQ ID NO. 10.

34. The purified toxin, according to claim 31, having a molecular weight of approximately 40-50 kDa.

35. A purified toxin active against a non-mammalian pest, wherein said toxin immunoreacts with an antibody to an approximately 40-50 kDa toxin, or a fragment thereof, from a *Bacillus thuringiensis* isolate selected from the group consisting of PS80JJ1, having the

identifying characteristics of NRRL B-18679; PS149B1, having the identifying characteristics of NRRL B-21553; and PS167H2, having the identifying characteristics of NRRL B-21554.

36. The purified toxin, according to claim 35, wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11.

37. The purified toxin, according to claim 36, having a molecular weight of about 40-50 kDa.

38. A purified toxin having activity against a non-mammalian pest, wherein said toxin is encoded by a nucleotide sequence wherein a portion of said nucleotide sequence can be amplified by PCR using a primer pair selected from the following group:

SEQ ID NOS. 20 and 24 to produce a fragment of about 495 bp; SEQ ID NOS. 20 and 25 to produce a fragment of about 594 bp; SEQ ID NOS. 21 and 24 to produce a fragment of about 471 bp; and SEQ ID NOS. 21 and 25 to produce a fragment of about 580 bp.

39. The purified toxin, according to claim 38, wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11.

40. The purified toxin, according to claim 39, having a molecular weight of about 40-50 kDa.

41. A purified toxin active against a non-mammalian pest, wherein said toxin comprises a pesticidal portion of the amino acid sequence shown in SEQ ID NO. 28.

42. The purified toxin, according to claim 41, wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11.

43. The purified toxin, according to claim 42, wherein said toxin comprises a pesticidal portion of the consensus sequence in Figure 1.

44. The purified toxin, according to claim 42, having a molecular weight of approximately 40-50 kDa.

45. A purified toxin active against a non-mammalian pest, wherein said toxin comprises an amino acid sequence which has at least about 75% homology with a pesticidal portion of an amino acid sequence selected from the group consisting of SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 38, and SEQ ID NO. 43.

46. The purified toxin, according to claim 45, wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11.

47. The purified toxin, according to claim 46, which comprises an amino acid sequence which has at least about 80% homology with a pesticidal portion of an amino acid sequence selected from the group consisting of SEQ ID NO. 11, SEQ ID NO. 13, and SEQ ID NO. 15, SEQ ID NO. 38, and SEQ ID NO. 43.

48. The purified toxin, according to claim 46, which comprises an amino acid sequence which has at least about 90% homology with a pesticidal portion of an amino acid sequence selected from the group consisting of SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 38, and SEQ ID NO. 43.

49. The purified toxin, according to claim 46, having a molecular weight of approximately 40-50 kDa.

50. A purified toxin active against a non-mammalian pest, wherein said toxin is encoded by a nucleotide sequence which hybridizes under stringent conditions with a nucleotide sequence selected from the group consisting of: DNA which encodes SEQ ID NO. 3; DNA which encodes SEQ ID NO. 5; and DNA which encodes SEQ ID NO. 7.

51. The purified toxin, according to claim 50, having a molecular weight of approximately 10-15 kDa.

52. A purified toxin active against a non-mammalian pest, wherein said toxin immunoreacts with an antibody to an approximately 10-15 kDa toxin, or a fragment thereof, from a *Bacillus thuringiensis* isolate selected from the group consisting of PS80JJ1, having the identifying characteristics of NRRL B-18679; PS149B1, having the identifying characteristics of NRRL B-21553; and PS167H2, having the identifying characteristics of NRRL B-21554.

53. The purified toxin, according to claim 52, having a molecular weight of about 10-15 kDa.

54. A purified toxin having activity against a non-mammalian pest, wherein said toxin is encoded by a nucleotide sequence wherein a portion of said nucleotide sequence can be amplified by PCR using the primer pair of SEQ ID NO. 29 and SEQ ID NO. 33.

55. The purified toxin, according to claim 54, having a molecular weight of about 10-15 kDa.

56. A purified toxin active against a non-mammalian pest, wherein said toxin comprises a pesticidal portion of an amino acid sequence selected from the group consisting of SEQ ID NO. 32, SEQ ID NO. 36, and SEQ ID NO. 41.

57. The purified toxin, according to claim 56, wherein said toxin comprises the amino acid sequence shown in SEQ ID NO. 32.

58. The purified toxin, according to claim 56, having a molecular weight of approximately 10-15 kDa.

59. A purified toxin active against a non-mammalian pest, wherein said toxin comprises an amino acid sequence which has at least about 75% homology with an amino acid sequence selected from the group consisting of SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of SEQ ID NO. 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of SEQ ID NO. 41.

60. The purified toxin, according to claim 59, which comprises an amino acid sequence which has at least about 80% homology with an amino acid sequence selected from the group consisting of SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of SEQ ID NO. 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of sequence IDS NO. 41.

61. The purified toxin, according to claim 59, which comprises an amino acid sequence which has at least about 90% homology with an amino acid sequence selected from the group

consisting of SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of SEQ ID NO. 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of sequence IDS NO. 41.

62. A biologically pure culture of a *Bacillus thuringiensis* isolate selected from the group consisting of PS149B1, having the identifying characteristics of NRRL B-21553; and PS167H2, having the identifying characteristics of NRRL B-21554; and mutants thereof which retain pesticidal activity.

63. The biologically pure culture, according to claim 62, wherein said *Bacillus thuringiensis* isolate is PS149B1, having the identifying characteristics of NRRL B-21553.

64. The biologically pure culture, according to claim 62, wherein said *Bacillus thuringiensis* isolate is PS167H2, having the identifying characteristics of NRRL B-21554.

65. A composition of matter for controlling coleopterans comprising a *Bacillus thuringiensis* isolate selected from the group consisting of PS149B1, having the identifying characteristics of NRRL B-21553; and PS167H2, having the identifying characteristics of NRRL B-21554; and mutants thereof which retain activity against coleopterans, in association with an agricultural carrier appropriate for use in controlling coleopterans.

66. A method for controlling a non-mammalian pest comprising contacting said pest with a pesticidal amount of a *Bacillus thuringiensis* isolate, or a toxin of said *Bacillus thuringiensis* isolate, wherein said isolate is selected from the group consisting of PS149B1, having the identifying characteristics of NRRL B-21553; and PS167H2, having the identifying characteristics of NRRL B-21554; and mutants thereof which retain pesticidal activity.

67. The method, according to claim 66, wherein said *Bacillus thuringiensis* isolate is PS149B1, having the identifying characteristics of NRRL B-21553.

68. The method, according to claim 66, wherein said *Bacillus thuringiensis* isolate is PS167H2, having the identifying characteristics of NRRL B-21554.

69. A method for controlling a non-mammalian pest which comprises contacting said pest with a pesticidal amount of a *Bacillus thuringiensis* toxin wherein said toxin has a characteristic selected from the group consisting of:

(a) said toxin is encoded by a nucleotide sequence which hybridizes under stringent conditions with a nucleotide sequence selected from the group consisting of: DNA which encodes SEQ ID NO. 2, DNA which encodes SEQ ID NO. 4, DNA which encodes SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, DNA which encodes SEQ ID NO. 11, SEQ ID NO. 12, DNA which encodes SEQ ID NO. 13, SEQ ID NO. 14, DNA which encodes SEQ ID NO. 15, DNA which encodes SEQ ID NO. 16, DNA which encodes SEQ ID NO. 17, DNA which encodes SEQ ID NO. 18, DNA which encodes SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, DNA which encodes a pesticidal portion of SEQ ID NO. 28, SEQ ID NO. 37, DNA which encodes SEQ ID NO. 38, SEQ ID NO. 42, and DNA which encodes SEQ ID NO. 43; and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11;

(b) said toxin immunoreacts with an antibody to an approximately 40-50 kDa pesticidal toxin, or a fragment thereof, from a *Bacillus thuringiensis* isolate selected from the group consisting of PS80JJ1 having the identifying characteristics of NRRL B-18679, PS149B1 having the identifying characteristics of NRRL B-21553, and PS167H2 having the identifying characteristics of NRRL B-21554, and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11;

(c) said toxin is encoded by a nucleotide sequence wherein a portion of said nucleotide sequence can be amplified by PCR using a primer pair selected from the group consisting of SEQ ID NOS. 20 and 24 to produce a fragment of about 495 bp, SEQ ID NOS. 20 and 25 to produce a fragment of about 594 bp, SEQ ID NOS. 21 and 24 to produce a fragment of about 471 bp, and SEQ ID NOS. 21 and 25 to produce a fragment of about 580 bp, and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11;

(d) said toxin comprises a pesticidal portion of the amino acid sequence shown in SEQ ID NO. 28, and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11;

(e) said toxin comprises an amino acid sequence which has at least about 75% homology with an amino acid sequence selected from the group consisting of SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 38, and SEQ ID NO. 43; and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11;

(f) said toxin is encoded by a nucleotide sequence which hybridizes under stringent conditions with a nucleotide sequence selected from the group consisting of DNA which encodes SEQ ID NO. 3, DNA which encodes SEQ ID NO. 5, and DNA which encodes SEQ ID NO. 7;

(g) said toxin immunoreacts with an antibody to an approximately 10-15 kDa pesticidal toxin, or a fragment thereof, from a *Bacillus thuringiensis* isolate selected from the group consisting of PS80JJ1 having the identifying characteristics of NRRL B-18679, PS149B1 having the identifying characteristics of NRRL B-21553, and PS167H2 having the identifying characteristics of NRRL B-21554;

(h) said toxin is encoded by a nucleotide sequence wherein a portion of said nucleotide sequence can be amplified by PCR using the primer pair of SEQ ID NO. 29 and SEQ ID NO. 33;

(i) said toxin comprises a pesticidal portion of an amino acid sequence selected from the group consisting of SEQ ID NO. 32, SEQ ID NO. 36, and SEQ ID NO. 41; and

(j) said toxin comprises an amino acid sequence which has at least about 75% homology with an amino acid sequence selected from the group consisting of SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of SEQ ID NO. 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of sequence IDS NO. 41.

70. The method, according to claim 69, wherein the full length of said toxin is approximately 40-50 kDa.

71. The method, according to claim 69, wherein said toxin is encoded by a nucleotide sequence which hybridizes under stringent conditions with a nucleotide sequence selected from the group consisting of: DNA which encodes SEQ ID NO. 2, DNA which encodes SEQ ID NO. 4, DNA which encodes SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, DNA which encodes

SEQ ID NO. 11, SEQ ID NO. 12, DNA which encodes SEQ ID NO. 13, SEQ ID NO. 14, DNA which encodes SEQ ID NO. 15, DNA which encodes SEQ ID NO. 16, DNA which encodes SEQ ID NO. 17, DNA which encodes SEQ ID NO. 18, DNA which encodes SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, DNA which encodes a pesticidal portion of SEQ ID NO. 28, SEQ ID NO. 37, DNA which encodes SEQ ID NO. 38, SEQ ID NO. 42, and DNA which encodes SEQ ID NO. 43; and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11.

72. The method, according to claim 69, wherein said toxin immunoreacts with an antibody to an approximately 40-50 kDa pesticidal toxin, or a fragment thereof, from a *Bacillus thuringiensis* isolate selected from the group consisting of PS80JJ1 having the identifying characteristics of NRRL B-18679, PS149B1 having the identifying characteristics of NRRL B-21553, and PS167H2 having the identifying characteristics of NRRL B-21554, and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11.

73. The method, according to claim 69, wherein said toxin is encoded by a nucleotide sequence wherein a portion of said nucleotide sequence can be amplified by PCR using a primer pair selected from the group consisting of SEQ ID NOS. 20 and 24 to produce a fragment of about 495 bp, SEQ ID NOS. 20 and 25 to produce a fragment of about 594 bp, SEQ ID NOS. 21 and 24 to produce a fragment of about 471 bp, and SEQ ID NOS. 21 and 25 to produce a fragment of about 580 bp, and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11.

74. The method, according to claim 69, wherein said toxin comprises a pesticidal portion of the amino acid sequence shown in SEQ ID NO. 28, and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11.

75. The method, according to claim 69, wherein said toxin comprises an amino acid sequence which has at least about 75% homology with an amino acid sequence selected from the group consisting of SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 38, and SEQ ID NO. 43; and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11.

76. The method, according to claim 69, wherein the full length of said toxin is approximately 10-15 kDa.

77. The method, according to claim 69, wherein said toxin is encoded by a nucleotide sequence which hybridizes under stringent conditions with a nucleotide sequence selected from the group consisting of DNA which encodes SEQ ID NO. 3, DNA which encodes SEQ ID NO. 5, and DNA which encodes SEQ ID NO. 7.

78. The method, according to claim 69, wherein said toxin immunoreacts with an antibody to an approximately 10-15 kDa pesticidal toxin, or a fragment thereof, from a *Bacillus thuringiensis* isolate selected from the group consisting of PS80JJ1 having the identifying characteristics of NRRL B-18679, PS149B1 having the identifying characteristics of NRRL B-21553, and PS167H2 having the identifying characteristics of NRRL B-21554

79. The method, according to claim 69, wherein said toxin is encoded by a nucleotide sequence wherein a portion of said nucleotide sequence can be amplified by PCR using the primer pair of SEQ ID NO. 29 and SEQ ID NO. 33.

80. The method, according to claim 69, wherein said toxin comprises a pesticidal portion of an amino acid sequence selected from the group consisting of SEQ ID NO. 32, SEQ ID NO. 36, and SEQ ID NO. 41.

81. The method, according to claim 69, wherein said toxin comprises an amino acid sequence which has at least about 75% homology with an amino acid sequence selected from the group consisting of SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of SEQ ID NO. 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of sequence IDS NO. 41.

82. The method, according to claim 69, wherein said pest is an insect.

83. The method, according to claim 82, wherein said insect is a coleopteran.

84. The method, according to claim 82, wherein said insect is a lepidopteran.

85. The method, according to claim 69, wherein said pest is a mite.

86. The method, according to claim 69, wherein said pest is corn rootworm.

87. The method, according to claim 69, wherein said toxin is encoded by DNA which hybridizes with SEQ ID NO. 2, SEQ ID NO. 10, SEQ ID NO. 37, or SEQ ID NO. 42, and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11.

88. The method, according to claim 69, wherein said toxin comprises the consensus sequence shown in Figure 1 and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11.

89. The method, according to claim 69, wherein said toxin comprises an amino acid sequence which has at least about 75% homology with a pesticidal portion of an amino acid sequence selected from the group consisting of SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 38, and SEQ ID NO. 43.

90. The method, according to claim 69, wherein said toxin comprises an amino acid sequence which has at least about 90% homology with a pesticidal portion of an amino acid sequence selected from the group consisting of SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 38, and SEQ ID NO. 43.

91. The method, according to claim 69, wherein said toxin comprises the consensus sequence shown in Figure 1.

92. The method, according to claim 69, wherein said toxin comprises an amino acid sequence which has at least about 75% homology with an amino acid sequence selected from the group consisting of SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of SEQ ID NO. 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of sequence IDS NO. 41.

93. The method, according to claim 69, wherein said toxin comprises an amino acid sequence which has at least about 90% homology with an amino acid sequence selected from

the group consisting of SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of SEQ ID NO. 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of sequence IDS NO. 41.

94. A method for controlling a non-mammalian pest wherein said method comprises contacting said pest with a first toxin wherein said toxin has a characteristic selected from the group consisting of:

(a) said toxin is encoded by a nucleotide sequence which hybridizes under stringent conditions with a nucleotide sequence selected from the group consisting of: DNA which encodes SEQ ID NO. 2, DNA which encodes SEQ ID NO. 4, DNA which encodes SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, DNA which encodes SEQ ID NO. 11, SEQ ID NO. 12, DNA which encodes SEQ ID NO. 13, SEQ ID NO. 14, DNA which encodes SEQ ID NO. 15, DNA which encodes SEQ ID NO. 16, DNA which encodes SEQ ID NO. 17, DNA which encodes SEQ ID NO. 18, DNA which encodes SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, DNA which encodes a pesticidal portion of SEQ ID NO. 28, SEQ ID NO. 37, DNA which encodes SEQ ID NO. 38, SEQ ID NO. 42, and DNA which encodes SEQ ID NO. 43; and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11;

(b) said toxin immunoreacts with an antibody to an approximately 40-50 kDa pesticidal toxin, or a fragment thereof, from a *Bacillus thuringiensis* isolate selected from the group consisting of PS80JJ1 having the identifying characteristics of NRRL B-18679, PS149B1 having the identifying characteristics of NRRL B-21553, and PS167H2 having the identifying characteristics of NRRL B-21554, and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11;

(c) said toxin is encoded by a nucleotide sequence wherein a portion of said nucleotide sequence can be amplified by PCR using a primer pair selected from the group consisting of SEQ ID NOS. 20 and 24 to produce a fragment of about 495 bp, SEQ ID NOS. 20 and 25 to produce a fragment of about 594 bp, SEQ ID NOS. 21 and 24 to produce a fragment of about 471 bp, and SEQ ID NOS. 21 and 25 to produce a fragment of about 580 bp, and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11;

(d) said toxin comprises a pesticidal portion of the amino acid sequence shown in SEQ ID NO. 28, and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11;

(e) said toxin comprises an amino acid sequence which has at least about 75% homology with a pesticidal portion of an amino acid sequence selected from the group consisting of SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 38, and SEQ ID NO. 43; and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11;

and further comprising contacting said pest with a second toxin having a characteristic selected from the group consisting of:

(f) said toxin is encoded by a nucleotide sequence which hybridizes under stringent conditions with a nucleotide sequence selected from the group consisting of DNA which encodes SEQ ID NO. 3, DNA which encodes SEQ ID NO. 5, and DNA which encodes SEQ ID NO. 7;

(g) said toxin immunoreacts with an antibody to an approximately 10-15 kDa pesticidal toxin, or a fragment thereof, from a *Bacillus thuringiensis* isolate selected from the group consisting of PS80JJ1 having the identifying characteristics of NRRL B-18679, PS149B1 having the identifying characteristics of NRRL B-21553, and PS167H2 having the identifying characteristics of NRRL B-21554;

(h) said toxin is encoded by a nucleotide sequence wherein a portion of said nucleotide sequence can be amplified by PCR using the primer pair of SEQ ID NO. 29 and SEQ ID NO. 33;

(i) said toxin comprises a pesticidal portion of an amino acid sequence selected from the group SEQ ID NO. 32, SEQ ID NO. 36, and SEQ ID NO. 41; and

(j) said toxin comprises an amino acid sequence which has at least about 75% homology with an amino acid sequence selected from the group consisting of SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of SEQ ID NO. 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of sequence IDS NO. 41.

95. The process, according to claim 94, wherein said pest is selected from the group consisting of insects and mites.

96. The process, according to claim 95, wherein said pest is a coleopteran.

97. The process, according to claim 94, wherein said first toxin has a full length of about 40-50 kDa and said second toxin has a full length of about 10-15 kDa.

98. A recombinant host transformed to express a toxin having activity against a non-mammalian pest wherein said toxin has at least one characteristic selected from the group consisting of:

(a) said toxin is encoded by a nucleotide sequence which hybridizes under stringent conditions with a nucleotide sequence selected from the group consisting of: DNA which encodes SEQ ID NO. 2, DNA which encodes SEQ ID NO. 4, DNA which encodes SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, DNA which encodes SEQ ID NO. 11, SEQ ID NO. 12, DNA which encodes SEQ ID NO. 13, SEQ ID NO. 14, DNA which encodes SEQ ID NO. 15, DNA which encodes SEQ ID NO. 16, DNA which encodes SEQ ID NO. 17, DNA which encodes SEQ ID NO. 18, DNA which encodes SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, DNA which encodes a pesticidal portion of SEQ ID NO. 28, SEQ ID NO. 37, DNA which encodes SEQ ID NO. 38, SEQ ID NO. 42, and DNA which encodes SEQ ID NO. 43; and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11;

(b) said toxin immunoreacts with an antibody to an approximately 40-50 kDa pesticidal toxin, or a fragment thereof, from a *Bacillus thuringiensis* isolate selected from the group consisting of PS80JJ1 having the identifying characteristics of NRRL B-18679, PS149B1 having the identifying characteristics of NRRL B-21553, and PS167H2 having the identifying characteristics of NRRL B-21554, and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11;

(c) said toxin is encoded by a nucleotide sequence wherein a portion of said nucleotide sequence can be amplified by PCR using a primer pair selected from the group consisting of SEQ ID NOS. 20 and 24 to produce a fragment of about 495 bp, SEQ ID NOS. 20 and 25 to produce a fragment of about 594 bp, SEQ ID NOS. 21 and 24 to produce a fragment of about 471 bp, and SEQ ID NOS.

21 and 25 to produce a fragment of about 580 bp, and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11;

(d) said toxin comprises a pesticidal portion of the amino acid sequence shown in SEQ ID NO. 28, and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11;

(e) said toxin comprises an amino acid sequence which has at least about 75% homology with a pesticidal portion of an amino acid sequence selected from the group consisting of SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 38, and SEQ ID NO. 43; and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11;

(f) said toxin is encoded by a nucleotide sequence which hybridizes under stringent conditions with a nucleotide sequence selected from the group consisting of DNA which encodes SEQ ID NO. 3, DNA which encodes SEQ ID NO. 5, and DNA which encodes SEQ ID NO. 7;

(g) said toxin immunoreacts with an antibody to an approximately 10-15 kDa pesticidal toxin, or a fragment thereof, from a *Bacillus thuringiensis* isolate selected from the group consisting of PS80JJ1 having the identifying characteristics of NRRL B-18679, PS149B1 having the identifying characteristics of NRRL B-21553, and PS167H2 having the identifying characteristics of NRRL B-21554;

(h) said toxin is encoded by a nucleotide sequence wherein a portion of said nucleotide sequence can be amplified by PCR using the primer pair of SEQ ID NO. 29 and SEQ ID NO. 33;

(i) said toxin comprises a pesticidal portion of an amino acid sequence selected from the group consisting of SEQ ID NO. 32, SEQ ID NO. 36, and SEQ ID NO. 41; and

(j) said toxin comprises an amino acid sequence which has at least about 75% homology with an amino acid sequence selected from the group consisting of SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of SEQ ID NO. 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of sequence IDS NO. 41.

99. The recombinant host, according to claim 98, wherein said host expresses a first toxin which has a characteristic selected from the group consisting of:

(a) said toxin is encoded by a nucleotide sequence which hybridizes under stringent conditions with a nucleotide sequence selected from the group consisting of: DNA which encodes SEQ ID NO. 2, DNA which encodes SEQ ID NO. 4, DNA which encodes SEQ ID NO. 6, SEQ ID NO. 8, SEQ ID NO. 10, DNA which encodes SEQ ID NO. 11, SEQ ID NO. 12, DNA which encodes SEQ ID NO. 13, SEQ ID NO. 14, DNA which encodes SEQ ID NO. 15, DNA which encodes SEQ ID NO. 16, DNA which encodes SEQ ID NO. 17, DNA which encodes SEQ ID NO. 18, DNA which encodes SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, DNA which encodes a pesticidal portion of SEQ ID NO. 28, SEQ ID NO. 39, DNA which encodes SEQ ID NO. 38, SEQ ID NO. 42, and DNA which encodes SEQ ID NO. 43; and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11;

(b) said toxin immunoreacts with an antibody to an approximately 40-50 kDa pesticidal toxin, or a fragment thereof, from a *Bacillus thuringiensis* isolate selected from the group consisting of PS80JJ1 having the identifying characteristics of NRRL B-18679, PS149B1 having the identifying characteristics of NRRL B-21553, and PS167H2 having the identifying characteristics of NRRL B-21554, and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11;

(c) said toxin is encoded by a nucleotide sequence wherein a portion of said nucleotide sequence can be amplified by PCR using a primer pair selected from the group consisting of SEQ ID NOS. 20 and 24 to produce a fragment of about 495 bp, SEQ ID NOS. 20 and 25 to produce a fragment of about 594 bp, SEQ ID NOS. 21 and 24 to produce a fragment of about 471 bp, and SEQ ID NOS. 21 and 25 to produce a fragment of about 580 bp, and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11;

(d) said toxin comprises a pesticidal portion of the amino acid sequence shown in SEQ ID NO. 28, and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11; and

(e) said toxin comprises an amino acid sequence which has at least about 75% homology with an amino acid sequence selected from the group consisting of SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 38, and SEQ

ID NO. 43; and wherein said toxin does not have the amino acid sequence shown in SEQ ID NO. 11;

and said host expresses a second toxin having a characteristic selected from the group consisting of:

(f) said toxin is encoded by a nucleotide sequence which hybridizes under stringent conditions with a nucleotide sequence selected from the group consisting of DNA which encodes SEQ ID NO. 3, DNA which encodes SEQ ID NO. 5, and DNA which encodes SEQ ID NO. 7;

(g) said toxin immunoreacts with an antibody to an approximately 10-15 kDa pesticidal toxin, or a fragment thereof, from a *Bacillus thuringiensis* isolate selected from the group consisting of PS80JJ1 having the identifying characteristics of NRRL B-18679, PS149B1 having the identifying characteristics of NRRL B-21553, and PS167H2 having the identifying characteristics of NRRL B-21554;

(h) said toxin is encoded by a nucleotide sequence wherein a portion of said nucleotide sequence can be amplified by PCR using the primer pair of SEQ ID NO. 29 and SEQ ID NO. 33;

(i) said toxin comprises a pesticidal portion of an amino acid sequence selected from the group consisting of SEQ ID NO. 32, SEQ ID NO. 36, and SEQ ID NO. 41; and

(j) said toxin comprises an amino acid sequence which has at least about 75% homology with an amino acid sequence selected from the group consisting of SEQ ID NO. 3, SEQ ID NO. 5, SEQ ID NO. 7, pesticidal portions of SEQ ID NO. 32, pesticidal portions of SEQ ID NO. 36, and pesticidal portions of sequence IDS NO. 41.

100. The recombinant host, according to claim 98, wherein said host is selected from the group consisting of plants, yeasts, and bacteria.

101. The isolated polynucleotide, according to claim 1, wherein said nucleotide sequence hybridizes with SEQ ID NO. 37.

102. The isolated polynucleotide, according to claim 1, wherein said nucleotide sequence hybridizes with SEQ ID NO. 42.

103. The purified toxin, according to claim 31, which is encoded by DNA which hybridizes with SEQ ID NO. 37.

104. The purified toxin, according to claim 31, which is encoded by DNA which hybridizes with SEQ ID NO. 42.

105. The isolated polynucleotide, according to claim 24, wherein said toxin comprises the amino acid sequence shown in SEQ ID NO. 36.

106. The isolated polynucleotide, according to claim 24, wherein said toxin comprises the amino acid sequence shown in SEQ ID NO. 41.

107. The purified toxin, according to claim 56, wherein said toxin comprises the amino acid sequence shown in SEQ ID NO. 36.

108. The purified toxin, according to claim 56, wherein said toxin comprises the amino acid sequence shown in SEQ ID NO. 41.

109. An isolated polynucleotide comprising a nucleotide sequence which encodes an approximately 10-15 kDa 80JJ1 toxin active against non-mammaliian pests, wherein said nucleotide sequence has been optimized for expression in plants.

110. The isolated polynucleotide, according to claim 109, wherein said polynucleotide comprises the sequence shown in SEQ ID NO. 44.

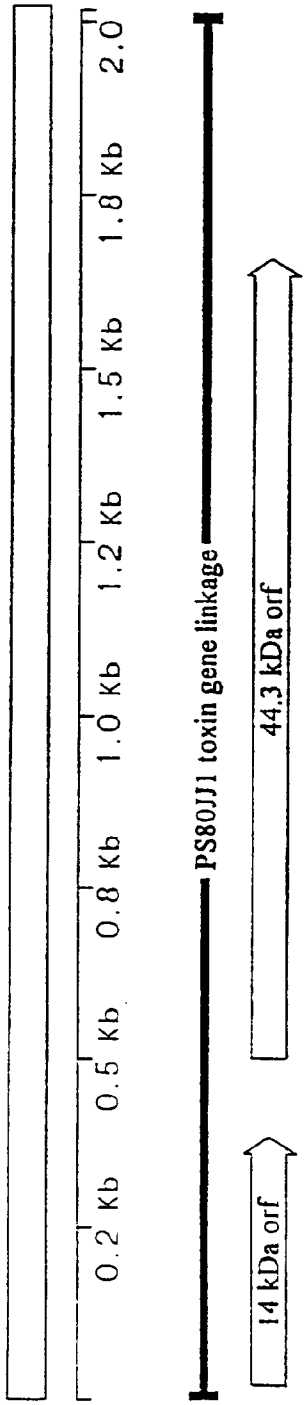
111. An isolated polynucleotide comprising a nucleotide sequence which encodes an approximately 40-50 kDa 80JJ1 toxin active against non-mammalian pests, wherein said nucleotide sequence has been optimized for expression in plants.

112. The isolated polynucleotide, according to claim 111, wherein said polynucleotide comprises the sequence shown in SEQ ID NO. 45.

FIG. 1

```
            1                                                    50
(149b145k)  .......... .....GLYAA TYLSLDDSGV SLMNKNDDDI DDYNLKWFLF
(167h245k)  .......... .......HAA TYLSLDDSGV SLMNKNDDDI DDYNLRWFLF
(80jj145k)  MLDTNKVYEI SNLANGLYTS TYLSLDDSGV SLMSKKDEDI DDYNLKWFLF
 Consensus  ---------- ---------- TYLSLDDSGV SLM-K-D-DI DDYNL-WFLF

            51                                                   100
(149b145k)  PIDDDQYIIT SYAANNCKVW NVNNDKINVS TYSSTNSIQK WQIKANGSSY
(167h245k)  PIDDNQYIIT SYAANNCKVW NVNNDKINVS TYSSTNSIQK WQIKANASSY
(80jj145k)  PIDNNQYIIT SYGANNCKVW NVKNDKINVS TYSSTNSVQK WQIKAKDSSY
 Consensus  PID--QYIIT SY-ANNCKVW NV-NDKINVS TYSSTNS-QK WQIKA--SSY

            101                                                  150
(149b145k)  VIQSDNGKVL TAGTGQALGL IRLTDESSNN PNQQWNLTSV QTIQLPQKPI
(167h245k)  VIQSNNGKVL TAGTGQSLGL IRLTDESPDN PNQQWNLTPV QTIQLPPKPT
(80jj145k)  IIQSDNGKVL TAGVGQSLGI VRLTDEFPEN SNQQWNLTPV QTIQLPQKPK
 Consensus  -IQS-NGKVL TAG-GQ-LG- -RLTDE---N -NQQWNLT-V QTIQLP-KP-

            151                                                  200
(149b145k)  IDTKLKDYPK YSPTGNIDNG TSPQLMGWTL VPCIMVNDPN IDKNTQIKTT
(167h245k)  IDTKLKDYPK YSQTGNIDKG TPPQLMGWTL IPCIMVNDPN IDKNTQIKTT
(80jj145k)  IDEKLKDHPE YSETGNINPK TTPQLMGWTL VPCIMVNDSK IDKNTQIKTT
 Consensus  ID-KLKD-P- YS-TGNI--- T-PQLMGWTL -PCIMVND-- IDKNTQIKTT

            201                                                  250
(149b145k)  PYYILKKYQY WQRAVGSNVA LRPHEKKSYT YEWGTEIDQK TTIINTLGFQ
(167h245k)  PYYILKKYQY WQQAVGSNVA LRPHEKKSYA YEWGTEIDQK TTIINTLGFQ
(80jj145k)  PYYIFKKYKY WNLAKGSNVS LLPHQKRSYD YEWGTEKNQK TTIINTVGLQ
 Consensus  PYYI-KKY-Y W--A-GSNV- L-PH-K-SY- YEWGTE--QK TTIINT-G-Q

            251                                                  300
(149b145k)  INIDSGMKFD IPEVGGGTDE IKTQLNEELK IEYSHETKIM EKY.......
(167h245k)  INIDSGMKFD IPEVGGGTDE IKTQLNEELK IEYSRETKIM EKY.......
(80jj145k)  INIDSGMKFE VPEVGGGTED IKTQLTEELK VEYSTETKIM TKYQEHSEID
 Consensus  INIDSGMKF- -PEVGGGT-- IKTQL-EELK -EYS-ETKIM -KY-------

            301                                                  350
(149b145k)  .......... .......... .......... .......... ..........
(167h245k)  .......... .......... .......... .......... ..........
(80jj145k)  NPTNQPMNSI GLLIYTSLEL YRYNGTEIKI MDIETSDHDT YTLTSYPNHK
 Consensus  ---------- ---------- ---------- ---------- ----------

            351                                  386
(149b145k)  .......... .......... .......... ......
(167h245k)  .......... .......... .......... ......
(80jj145k)  EALLLLTNHS YEEVEEITKI PKHTLIKLKK HYFKK.
 Consensus  ---------- ---------- ---------- -----.
```

FIG. 2


0.2 Kb
0.5 Kb
0.8 Kb
1.0 Kb
1.2 Kb
1.5 Kb
1.8 Kb
2.0
PS80JJ1 toxin gene linkage
14 kDa orf
44.3 kDa orf